JOURNAL OF AGRICULTURAL RESEARCH

VOL. 34 WASHINGTON, D. C., APRIL 1, 1927 NO. 7

PRELIMINARY STUDIES ON THE RELATION OF FIRE INJURY TO BARK-BEETLE ATTACK IN WESTERN YELLOW PINE[1]

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INTRODUCTION

That the effect of fire in pine forests of the Pacific slope region has been studied in many of its aspects is indicated by the published information on this problem. One phase, which has been only briefly discussed in the literature now available, is that of bark-beetle infestations which follow in the wake of fires.[2]

It has been generally recognized that there is a direct relation between fire injury and subsequent insect damage on burned-over areas. Two types of loss are involved—destruction of the merchantable value of fire-killed trees by wood-boring insects, and the actual killing by bark beetles of trees that survived the fire.

An increase in the number of trees infested by bark beetles is a frequent aftermath of the so-called "light" fires which rarely kill mature trees. A very perceptible flare-up of the infestation in a burned-over area frequently follows a fire of this character. Many trees only moderately injured by fire and apparently capable of recovering will succumb to the attacks of these beetles.

Such damage follows as a result of a sporadic local increase of bark-beetle population within the fire area which can be explained only by the assumption that numbers of beetles fly into the area from the surrounding forest. Because of fire injury certain trees become especially attractive to the beetles. The physiological basis for this attraction is but vaguely understood. The odor of fire-scorched foliage and cambium may be an attractive influence, or the insects themselves may possess an instinctive ability to select those trees in which sap resistance has been weakend by fire injury, but whatever the influence, it is evidently a very strong one in determining the behavior of Dendroctonus beetles.

The multifold aspects of the interrelation of fire and insect damage raise many questions that are pertinent to the protection of pine forests. What type and what degree of fire injury make trees attractive to bark beetles? Are such trees capable of recovery if not attacked by insects? Do bark beetles "breed up" in fire areas, increasing their numbers to an epidemic status, and then become aggressive in uninjured trees in and around burned areas? The answers to these questions can be found only by a careful study and analysis of the infestations which develop within and adjacent to

[1] Received for publication Oct. 12, 1926; issued May, 1927.

[2] SHOW, S. B., and KOTOK, E. I. THE RÔLE OF FIRE IN THE CALIFORNIA PINE FORESTS. U. S. Dept. Agr. Bul. 1294, 80 p., illus. 1924.

such areas. Since 1917 the Bureau of Entomology has had studies of this character under way in the yellow-pine regions of California and Oregon.

The results now available indicate the tendencies to be expected under the conditions where these fires occurred. Rarely do any two fires occur under identical conditions, and each presents its own individual complex of factors which may modify results. It can not be claimed therefore that the results which are given in this paper will be found applicable to all fires under any conditions; they are intended only to serve as a contribution toward the information which is being accumulated on this problem.

THE MISTLETOE BURN

GENERAL CONDITIONS OF THE AREA

One area in which the more intensive studies have been carried on is known as the Mistletoe burn in southern Oregon. This fire, which occurred October 31, 1917, burned over 800 acres on the slopes of the Siskiyou Mountains, 7 miles southeast of the town of Ashland. The elevations within the burn range from 3,000 to 4,000 feet above sea level, with exposures of the slopes mainly to the south and east. The timber type consists of a mixed stand of yellow pine (*Pinus ponderosa*) and Douglas fir (*Pseudotsuga taxifolia*), with a small amount of sugar pine (*Pinus lambertiana*) intermixed. At the lower elevations and on the poorer sites brush cover predominated, which graded into fairly well-stocked mature stands of conifers on the more favorable slopes. There was abundant reproduction and new growth of conifers on the better sites.

From a 100 per cent cruise of the burned-over area, the volume of merchantable timber of the more important species was found to be as follows: Yellow pine, 44.5 per cent; Douglas fir, 52.5 per cent; sugar pine, 3 per cent.

STUDIES OF AREA PRECEDING FIRE

Both the area that was burned and the surrounding forest had been under observation since 1914. In a study begun in 1914 to determine the progress of the bark-beetle infestation in the general region, annual cruises had been made in which all insect-killed yellow pine and sugar pine were marked and records taken from each tree. Although the fire started unexpectedly from unknown origin, the status of the bark-beetle infestation at the time of the fire was definitely known.

CHARACTER OF THE FIRE

In the open and mature stands the damage was typical of a ground fire of moderate severity. Seedlings and small reproduction were killed, a few butt scars were newly formed on the larger trees, and old scars were enlarged, but no yellow pine trees of merchantable size were killed outright.

On the steeper slopes, however, the fire developed considerable intensity in the thickets of new growth and in brush cover and either killed or consumed all the forest cover. In these sections of the area, where brush and trees of pole sizes were intermixed, the fire reached the crowns, burned away the foliage, and killed outright a considerable part of the stand.

CRUISE OF FIRE DAMAGE

The following year (1918) a cruise was made of the entire burn to determine the amount of damage to merchantable trees. As a basis for a study of subsequent insect attacks, all trees on the area were marked according to the nature of fire injury. A base line was run across the burn and the entire area was covered by strips, one tally wide, run at right angles to this base line. All trees over 10 inches d. b. h. were examined and classified according to the degree of fire injury and were marked for future reference according to class of injury. This was done by smoothing off the outer bark with an ax and writing the number of the class with a black crayon. At the same time cruises were made in the surrounding areas and the loss was analyzed by zones from 1 to 6 miles distant from the center of the burn. A similar cruise was made in November, 1919, and a final one in 1920 (fig. 1).

LIMITATIONS OF DATA

This study was planned to determine the relation of the fire-injured trees to subsequent bark-beetle attack and it was found unnecessary to consider trees under 10 inches d. b. h. No quantitative data were taken on the destruction of seedlings, reproduction, brush, and other cover not of a merchantable character, although this loss was a factor in the total amount of damage. Douglas fir also was thrown out of consideration when it was found that the only insects which attacked it were secondary borers and that their attacks were confined largely to fire-killed trees. The percentage of sugar pine was so small as to be of practically no importance. The study, therefore, is confined entirely to the infestation of the western pine beetle (*Dendroctonus brevicomis* Lec.), which developed in the fire-injured western yellow pine. In discussing the losses caused by this beetle, only those trees killed by its attacks are considered.

TABLE 1.—*Yellow pine on Mistletoe burn classified according to fire injury*

Class	Number of trees	Per cent	Volume (board feet)	Per cent
Class 1, uninjured by fire	1,577	65.7	1,385,430	64.7
Class 2, light fire injury	573	23.9	539,370	25.2
Class 3, medium fire injury	113	4.7	120,360	5.6
Class 4, heavy fire injury	57	2.4	80,450	3.7
Class 5, killed by fire	80	3.3	16,720	.8
Total	2,400	100.0	2,142,330	100.0

Trees in classes 2, 3, and 4 are those which survived the fire but with different degrees of injury (Table 1). In all three classes the bole of the tree was more or less blackened, and in classes 3 and 4 the cambium also was damaged. In class 2 the foliage was partly scorched, causing the needles to discolor. In class 3 practically all of the crown was scorched, but not sufficiently to kill the terminal buds on lateral limbs and top. In class 4 the entire foliage was burned away and the terminal buds killed, but the cambium still remained green at the time of examination six months after the fire. In class 5 an examination near the base showed that the cambium had been deadened by the first effects of the fire, so that these trees were unquestionably fire killed. Figure 2 illustrates the comparative degrees of fire injury used as a basis for this classification. Photographs of trees in classes 3 and 4 are shown in Figure 3.

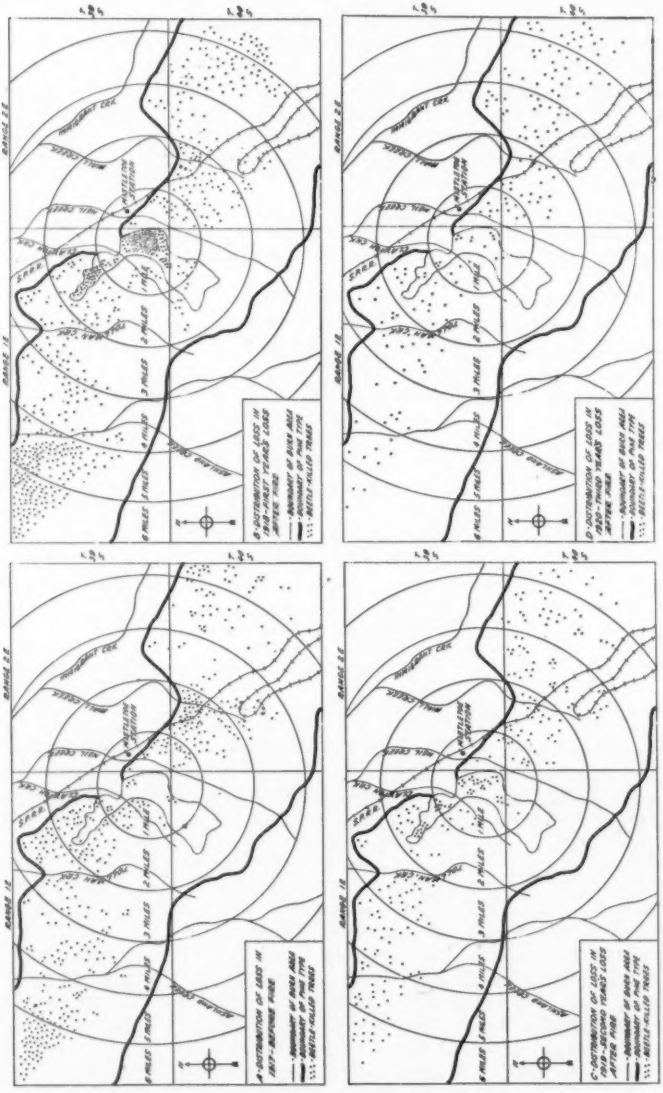

FIG. 1.—Distribution of trees killed by bark beetles (*Dendroctonus brevicomis*) in the Mistletoe burn and in the surrounding zones one year before and three years after the fire

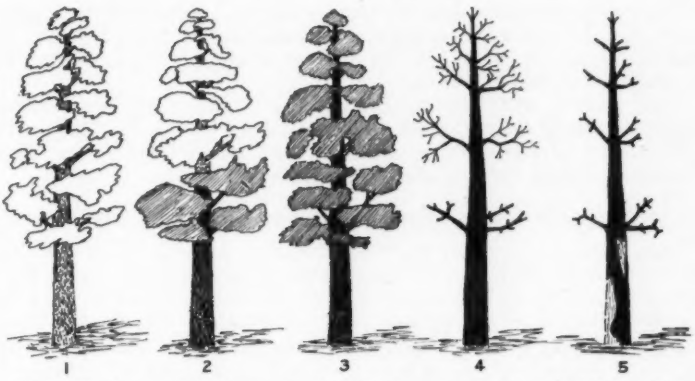

FIG. 2.—Five trees, each illustrating one of the five classes represented on the Mistletoe burn: 1, Uninjured by fire; 2, light fire injury; 3, medium fire injury; 4, heavy fire injury; 5, killed by fire

FIG. 3.—Fire-injured trees which attract bark-beetle attacks. A, class 3. Two fire-injured trees, showing base and middle portions of trunks fire charred and almost all of foliage scorched. The buds of the new needle growth were uninjured toward the top of the tree. The larger trees suffering this degree of fire injury have some chance to recover if not attacked by insects. They are, however, more attractive to bark beetles than trees in any other class. Over 80 per cent of the trees in this class were attacked the first season after the fire. B, class 4. A group of three small trees showing heavy fire damage. The trunks of these trees were scorched for their entire length, and the greater part of the foliage was burned away. The terminal buds were killed, although the cambium remained green until the following season. This class is attractive to bark beetles only during the first season after the fire. Trees injured to this extent eventually die as a result of the fire injury, even though they escape bark-beetle attack

Butt scars were considered, but as few of these were formed by this fire and no trees so injured were attacked by bark beetles, this class of damage has been left out of consideration in order to simplify the analysis of other factors.

Trees entirely killed by the fire average considerably smaller than those composing the other classes, as is shown by the fact that while 3.3 per cent of the number of trees were killed by the fire, these amounted to only 0.7 per cent of the volume.

VOLUME OF TIMBER KILLED BY BARK BEETLES WITHIN THE BURN

From May 1 to October 31, 1917, the season preceding the fire, the western pine beetle killed 20 yellow pine trees within the area of the burn, containing a volume of 12,500 board feet (Table 2). No attacks occurred in November, 1917, following the fire. This is explained by the fact that at that time the attacks of the overwintering broods had already occurred and beetles would not fly again until the following spring.

During 1918 attacks occurred in many trees within the burn. Wherever fire-injured yellow pine predominated, heavy centers of infestation formed. The volume killed within the burn amounted to 200 board feet per acre, whereas in the surrounding area for a distance of 6 miles the loss averaged 29 board feet per acre. During 1918 a volume of 159,720 board feet was destroyed within the burn—an increase over 1917 of 1,000 per cent.

In 1919 the loss was reduced to 42,180 board feet. This represents a reduction of about 74 per cent. In 1920 the loss was further reduced to 15,680 board feet.

The course of the infestation within the burn was marked by a very pronounced increase during the season following the fire and a subsidence within the two following seasons to a point about as low as that which existed before the fire.

VOLUME OF TIMBER KILLED BY BARK BEETLES ON AREA SURROUNDING BURN

Outside of the burned area the volume of infested timber followed a course during the seasons of 1918, 1919, and 1920 quite different from that within the burn. The yellow pine type of this locality consists of a strip extending along the slopes of the Siskiyou Mountains between the elevations of 2,000 and 5,000 feet. Open valleys predominate below these elevations, while above 5,000 feet the forest type is composed almost entirely of fir and subalpine species. As a result of this type distribution, the area susceptible to pine-beetle infestation consists of a narrow belt from 2 to 3 miles in width extending from northeast to southwest.

The relation of the surrounding zones to the fire area is shown in Figure 1. The fire area formed an irregular L-shaped strip approximately 3½ miles long by one-half mile wide. The axis of this area was assumed to be the center of the burn. From this point the first zone was defined as a circle with a 1-mile radius. The second zone was drawn from the same center with a 2-mile radius, and included the area within the outer limit of this circle and the outer boundary of the first zone. The third, fourth, fifth, and sixth zones were laid out concentrically in the same manner.

TABLE 2.—*Progress of bark-beetle infestation on fire area and on surrounding area*

Year	Fire area, 800 acres		Surrounding area, 18,500 acres	
	Number of trees	Volume (board feet)	Number of trees	Volume (board feet)
1917	20	12,500	449	403,670
1918	220	159,720	508	516,130
1919	50	42,180	224	199,210
1920	17	15,680	181	160,980
Total	307	230,080	1,362	1,279,990

The yellow pine type, however, extended in the form of a long narrow strip about 2½ miles in width along the flanks of the Siskiyou Mountains, so that only part of the area in each zone was covered with timber susceptible to attack by the western pine beetle. The area of pine type in each zone, therefore, was more or less irregular and does not conform to the total areas of the concentric circles defining the limits of the zones. However, it is the distance to which the effects of the fire influenced the infestation in the surrounding pine area that is the important factor under consideration, and this is definitely determined by the boundaries of the various zones established.

All of the area included within these zones had been cruised before the fire, the insect-killed trees had been marked and located by map, and individual tree records taken. This same system of marking was continued for three years after the fire. The data thus collected were compiled according to the zone in which the loss occurred.

The records thus obtained show that for the general area surrounding the burn there was a slight tendency toward increase of infestation during the season of 1918, but at the same time the zones within 3 miles of the burn registered a perceptible decrease. During 1919, however, when the volume of infestation within the burn decreased 74 per cent, the first zone immediately adjoining showed an increase of nearly 60 per cent, while in all zones beyond this there was a very marked decrease during the season (Tables 3 and 4).

TABLE 3.—*Number of trees killed and loss in board feet in fire area and in each surrounding zone from 1917 to 1920, inclusive*

Fire area and surrounding zones	Number of trees killed and volume destroyed							
	1917		1918		1919		1920	
	Trees	Volume (board feet)	Trees	Volume (board feet)	Trees	Volume (board feet)	Trees	Volume (board feet)
Fire area	20	12,500	220	159,720	50	42,180	17	15,680
Zone 1	47	42,255	31	31,500	56	49,800	30	26,680
Zone 2	122	109,680	104	105,665	44	39,120	38	33,820
Zone 3	82	73,720	74	75,180	47	41,800	42	37,350
Zone 4	68	61,135	90	91,440	41	36,450	38	33,780
Zone 5	71	63,830	129	131,065	22	19,580	30	26,680
Zone 6	59	53,050	80	81,280	14	12,460	3	2,670
Total	469	416,170	728	675,850	274	241,390	198	176,660

TABLE 4.—*Annual loss per acre on the fire area and on each of the surrounding zones for one year previous to fire and for three years after*

Fire area and surrounding zones	Acreage of yellow pine type in each zone	Board-foot loss per acre			
		1917	1918	1919	1920
Fire area	800	16	200	53	20
Zone 1	920	46	34	54	29
Zone 2	3,440	32	31	11	10
Zone 3	4,960	15	15	8	8
Zone 4	4,000	15	23	9	8
Zone 5	3,240	20	40	6	8
Zone 6	1,200	44	68	10	2

From this evidence it would appear that the natural tendency of the infestation was to increase during 1918 and to decrease during 1919. These natural tendencies were disturbed, however, by the attractiveness to the beetles of the fire-injured trees in 1918, which caused many beetles to leave the first three surrounding zones and concentrate in the burn. In 1919, as the attraction of the fire-injured trees had subsided, some of the beetle population which had concentrated in the burn attacked trees in the area immediately adjoining, causing a perceptible increase locally although all other areas registered a decrease. A comparison of the course of the infestation both inside and outside of the burn is shown in Figure 4 and Table 4.

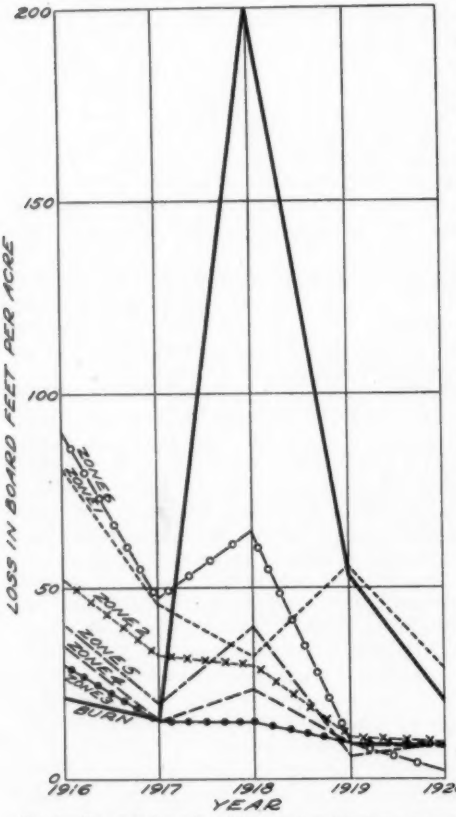

FIG. 4.—Comparative rates of loss, in board feet per acre, of timber killed by bark beetles on burned area and on each of the surrounding zones during the season preceding the fire and for three seasons subsequent to it (Mistletoe burn)

CLASSES OF FIRE-INJURED TREES ATTACKED

The selection made by the beetles in the various classes of fire-injured trees is shown in Figure 5, A. There was a decided tendency on the part of the beetles to select trees in classes 2 and 3 which sustained light to medium fire injury. Some attacks occurred in trees of class 1 which were uninjured in any way by the fire, but it is evident that trees which sustained a light to medium fire injury were far more attractive to the beetles than any other trees on the burned area. These were trees which appeared to be likely to recover from the effects of the fire itself.

TABLE 5.—*Number of trees of each class attacked and loss in board feet during 1918, 1919, and 1920*

Class	1918 (first year after fire)		1919 (second year after fire)		1920 (third year after fire)	
	Trees	Volume (board feet)	Trees	Volume (board feet)	Trees	Volume (board feet)
1 (uninjured by fire)	17	24,370	21	16,050	12	8,560
2 (light fire injury)	81	60,140	11	8,260	3	2,620
3 (medium injury)	84	49,910	18	17,170	2	4,500
4 (heavy injury)	38	25,300	0	0	0	0
5 (fire-killed)	0	0	0	0	0	0
Total	220	159,720	50	41,480	17	15,680

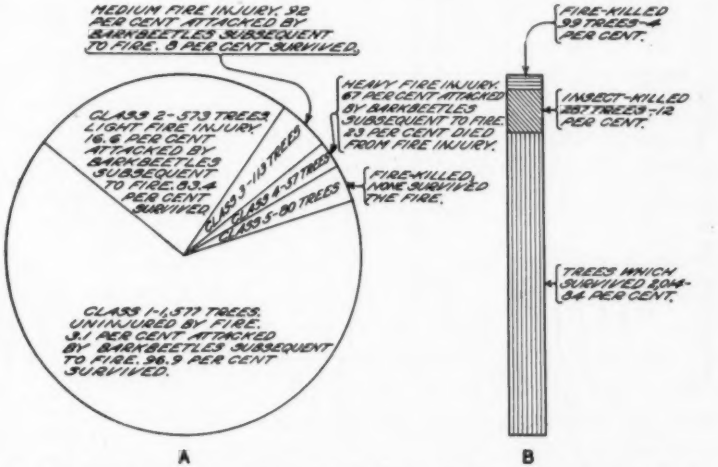
MEDIUM FIRE INJURY. 92 PER CENT ATTACKED BY BARKBEETLES SUBSEQUENT TO FIRE. 8 PER CENT SURVIVED.
CLASS 2-573 TREES. LIGHT FIRE INJURY. 16.6 PER CENT ATTACKED BY BARKBEETLES SUBSEQUENT TO FIRE. 83.4 PER CENT SURVIVED.
CLASS 3-113 TREES
CLASS 4-37 TREES
CLASS 5-90 TREES
CLASS 1-1,577 TREES. UNINJURED BY FIRE. 3.1 PER CENT ATTACKED BY BARKBEETLES SUBSEQUENT TO FIRE. 96.9 PER CENT SURVIVED.
HEAVY FIRE INJURY. 67 PER CENT ATTACKED BY BARKBEETLES SUBSEQUENT TO FIRE. 33 PER CENT DIED FROM FIRE INJURY.
FIRE-KILLED, NONE SURVIVED THE FIRE.
FIRE-KILLED 99 TREES-4 PER CENT.
INSECT-KILLED 287 TREES-12 PER CENT.
TREES WHICH SURVIVED 2,014-84 PER CENT.
A
B

FIG. 5.—Relative amounts of fire and insect damage following the Mistletoe fire. A, relative number of trees in each class of fire injury and percentage of each class attacked by bark beetles; B, percentages of surviving, insect-killed, and fire-killed trees

TABLE 6.—*Number of trees of each class attacked and percentage of annual infestation in each class, during 1918, 1919, and 1920*

Class	1918		1919		1920		Total for period	
	Number of trees	Percentage of infestation	Number of trees	Percentage of infestation	Number of trees	Percentage of infestation	Number of trees	Percentage of infestation
1	17	8	21	42	12	71	50	17.5
2	81	37	11	22	3	17	95	33
3	84	38	18	36	2	12	104	36
4	38	17	0	0	0	0	38	13
5	0	0	0	0	0	0	0	0
Total each year	220	100	50	100	17	100	287	100

PERIOD OF ATTRACTION TO TREES WITHIN THE BURN

Trees in classes 1, 2, and 3 were attacked during each of the three years following the fire. (Tables 5 and 6 and Fig. 6.) In classes 2 and 3 by far the greater part of these attacks, or more than 80 per cent, occurred during the first year after the fire. During the second and third years the attacks in trees of these classes were still relatively greater than the attacks in uninjured trees but to a much less extent than during the first season.

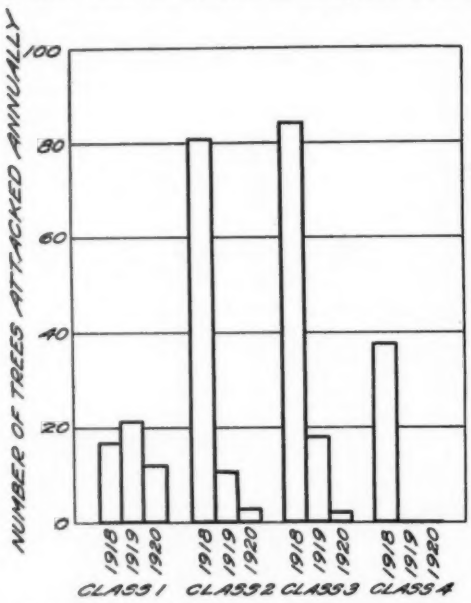

FIG. 6.—Relative number of trees attacked annually on Mistletoe burn by bark beetles in each class of fire injury for three seasons subsequent to fire

All attacks in class 4 occurred during the first season following the fire. The fact that no attacks occurred during the second and third seasons after the fire is readily explained, as all trees in this class that were not attacked by beetles during the first season died as a result of fire injury before the period of attack during the second season.

While the period of attraction to fire-injured trees was extended during the second and third seasons, the main concentration of beetles within the burn occurred during the first season after the fire.

DEVELOPMENT OF BROODS IN FIRE-INJURED TREES

EFFECT OF FIRE ON BROODS IN OUTER BARK OF INFESTED TREES

There were six yellow pine trees infested with broods of the western pine beetle standing within the burn at the time that the fire occurred. The broods in these trees consisted mainly of full-grown larvae and a few pupae in the outer bark. All of these trees were exposed to heat from a ground fire of moderate intensity, and the outer bark near the base received more or less scorching. In five of these trees the broods continued to develop after the fire and a normal emergence occurred from them the following spring. In one tree, however, the broods in larval and pupal stages were killed on one side of the trunk near the base where the heat had been severe.

MORTALITY OF BROODS IN TREES ATTACKED AFTER FIRE

During the season of 1918 it was found that many broods of the western pine beetle in the fire-scorched trees were dying in the early

larval stages. In trees where this high mortality occurred it was noted that an extremely moist condition of the inner bark was prevalent. The cause of this excess of moisture has not been definitely determined. Apparently it resulted from the sudden cutting off of the respiratory functions of the tree due to the defoliation caused by fire. Much of the moisture in the sapwood and cambium condensed in the inner bark, resulting in conditions highly unfavorable to the young larvae of the western pine beetle. The term used by cruisers to designate this condition is "sour sap."

In order to obtain some idea of the extent of this mortality, a study was made of the number of beetles attacking the bark surface of these sour-sap trees as compared with the subsequent progeny which emerged. A total of 272 square feet of bark was collected from 28 trees which had been attacked and abandoned by the beetles, or an average of nearly 10 square feet to the tree. An effort was made to select fairly representative trees, and the bark sections were collected at different heights from each tree ranging from 6 to 70 feet from the base.

The holes through which pairs of beetles entered the cambium were counted, as were also the exit holes from the same bark sections. Previous studies had determined that the number of beetles going in through each entrance hole averaged 2.59. Upon this basis it was possible to arrive at the following comparison of the number of beetles that entered and the number that emerged from fire-injured trees.

Number of square feet of bark analyzed	272
Total number of entrance holes	1,372
Total number of beetles attacking (1,372×2.59)	3,553
Total number of progeny emerging (exit holes)	3,277
Reduction in emergence over attack	276

This analysis indicates that although the fire-injured trees attracted beetles to such an extent as to cause a concentration within the burn, at the same time they afforded a very unfavorable breeding ground for the insects and in the end contributed toward an actual reduction of their numbers.

DEVELOPMENT OF BROODS IN TREES ATTACKED OUTSIDE OF BURN

A similar study was made of bark from trees outside of the burn in order to compare the mortality of the beetles in fire-injured trees with that of beetles in normally infested trees. A total of 341 square feet of bark was collected from 76 trees. The results of these counts were as follows:

Number of square feet of bark analyzed	341
Total number of entrance holes	2,048
Total number of beetles attacking (2,048×2.59)	5,294
Total number of progeny emerging (exit holes)	22,807
Excess of emergence over attack	17,513

A direct comparison of the average attacks and emergence per square foot unit on fire-injured and normal trees is as follows:

On fire-injured trees: Five entrance holes; 13 beetles attacking, 12 emerging; that is, a decrease of 1 beetle, or 7.7 per cent.

On normal trees: Six entrance holes; 16 beetles attacking, 67 emerging; that is, an increase of 51 beetles, or 318.8 per cent.

A graphic comparison is also shown in Figure 7. In normal trees there was an increase during 1918 of approximately 320 per cent, whereas in the fire-injured trees there was an actual decrease of about 8 per cent. To this extent the fire acted as a controlling factor by reducing the number of beetles that were attracted into the burned area.

SUMMARY OF RESULTS ON MISTLETOE BURN

Fire Damage

Of the total merchantable pine volume on the area covered by this burn, 0.7 per cent was immediately killed by the fire; 3.7 per cent was scorched to the point of complete defoliation, so that recovery was improbable; 30.6 per cent had the foliage partially scorched, but apparently was capable of recovery; the remaining 65 per cent was not visibly injured by the fire.

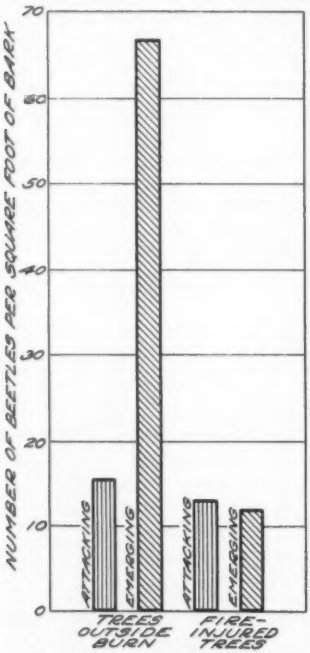

FIG. 7.—Comparison of brood development in trees under normal conditions adjacent to Mistletoe burn and in fire-injured trees

Insect Damage

This fire, which occurred late in October, produced no disturbance of the bark-beetle infestation in and around the area during the remainder of that season.

Throughout the season following the burn, fire-injured trees formed an attraction which caused a concentration of beetles in the burned-over area. As a result, the volume of infested timber within the burn increased 1,000 per cent over that on the same area during the preceding year.

The attraction of the beetles to individual trees was largely determined by the character of the fire injury. A decided preference was shown for trees in which the lower trunk and foliage had been moderately or lightly scorched. This attraction was most pronounced during the first year after the fire.

Influence of Fire Upon Subsequent Infestation

This concentration of beetles within the burn did not cause an epidemic infestation that continued to increase within the burn or to spread to surrounding areas. During the second year after the fire the infestation both in the burn and in the surrounding area returned to a status approaching that which existed before the fire.

The volume of infestation in the surrounding area was apparently influenced by developments within the burn. During the year following the fire the volume of infestation in the zones immediately adjoining and within 3 miles of the burn was reduced, apparently because of the attraction of beetles to the fire-injured trees in the burned-over area. The infestation in zones more than 3 miles distant from the burn increased slightly during the same season.

A study of bark collected from beetle-attacked fire-injured trees showed that on account of excessive moisture in the inner bark a heavy mortality occurred in the western pine beetle broods. A comparison indicates that the number of beetles emerging from fire-injured trees was 8 per cent less than the number of parent adults which attacked, resulting in a slight reduction of the second generation of the beetle population in the burned area.

THE SISKIYOU BURN

The Siskiyou burn was studied in the same detail as the Mistletoe burn just described. The fire occurred on August 15, 1918, and covered about 200 acres situated approximately 4 miles south of the Mistletoe unit.

Type and slope conditions were quite similar to those on the Mistletoe burn, but the fire was of somewhat greater severity as shown by the correspondingly greater damage to mature trees. Cruises were made in which the fire-injured trees were classified on the same basis as in the Mistletoe burn (Table 7).

TABLE 7.—*Yellow pine on Siskiyou burn classified according to fire injury*

Class	Number of trees	Per cent
1 (uninjured by fire)	176	31
2 (light fire injury)	205	37
3 (medium fire injury)	52	9
4 (heavy fire injury)	114	20
5 (killed by fire)	18	3
Total	565	100

At the time of the fire there were two yellow pine trees with a volume of 260 feet which had been infested by the western pine beetle during the early summer preceding the fire. These trees represented the first seasonal generation and were nearly abandoned at the time. Within a few days after the fire there was a concentration of beetles on the injured trees in the burn. In this respect the effects of the Siskiyou fire differ from those of the Mistletoe area, where the beetles did not concentrate until the season following the fire. This difference is easily explained by the fact that the Siskiyou fire occurred during the seasonal flight period of the beetles while the Mistletoe fire occurred in the fall after this activity had subsided.

During the latter part of August and through September the beetles killed 41 new trees having a volume of 44,130 board feet, an increase of 1,950 per cent over the number killed by the preceding generation (Table 8).

TABLE 8.—*Loss from infestation of the western pine beetle in the Siskiyou burn*

Season of attack	Number of trees	Volume (board feet)	Increase or decrease in number of trees attacked	
			Increase	Decrease
			Per cent	*Per cent*
1918, first generation before fire	2	260		
1918, second generation after fire	41	44,130	1,950	
1919	62	74,990	51	
1920	4	3,150		93.5

During 1919 the insects continued to concentrate in the fire area and the loss continued, increasing 51 per cent over that of 1918. In 1920, however, the third year after the fire, there was a pronounced decline in attack and the loss for that season averaged 93.5 per cent less than that for 1919.

Table 8 shows that the infestation on this burn increased after the fire in about the same degree as that on the Mistletoe burn and that the cycle of increase and decrease was of practically the same duration. The heavy increase immediately after the fire was followed by an equally pronounced decrease within two seasons.

This infestation within the burn was distributed in the different classes of trees as shown in Table 9.

TABLE 9.—*Distribution of infestation in different classes of trees in the Siskiyou burn*

Class	1918			1919			1920		
	Number of trees	Volume (board feet)	Per cent of total volume	Number of trees	Volume (board feet)	Per cent of total volume	Number of trees	Volume (board feet)	Per cent of total volume
1	2	1,110	2.5	2	2,370	3	0	0	
2	8	8,680	19.7	15	13,050	17	0	0	
3	6	11,260	25.5	6	2,250	3	4	3,150	100
4	19	4,790	10.9	39	57,320	77	0	0	
5	6	18,290	41.4	0	0	0	0	0	
Total	41	44,130	100.0	62	74,990	100	4	3,150	100

COURSE OF INFESTATION IN SURROUNDING AREAS

The Siskiyou fire was so located that a study of the surrounding infestation by zones was not practicable. However, the general course of the infestation in the surrounding region can be determined by the figures showing the same progress in the area surrounding the Mistletoe burn.

No study was made of brood mortality in trees either within or without this fire area, but as the effects of the fire differ but little from those on the Mistletoe burn, there is reason to believe that the same high mortality in fire-injured trees resulted.

THE CHINQUAPIN BURN

Another area, which was studied in less detail than either of the others, is the Chinquapin burn, which is located 26 miles southeast of Ashland, Oreg. This fire occurred in September, 1915, and

covered 220 acres. The character of this fire was practically the same as that on the Mistletoe and Siskiyou burns.

The attractive influence of the fire-injured trees on this burn was most marked during 1916, the year following the fire. During this season there was a concentration of the western pine beetle within the area, resulting in an increase of 475 per cent over that on the area in 1915. Most of the attacks were in trees which had been scorched by the fire. While the infestation within the burn increased 475 per cent during this season, throughout the surrounding area it decreased 68 per cent.

In 1917 the infestation decreased both within the burn and in the surrounding area. This was followed in 1918 by an increase both within the burn and the surrounding area.

On the whole, the data from this area indicate that the main effect of this fire, the concentration of beetles within the fire area, had disappeared by the close of the second season after the fire.

EFFECTS OF CONTROLLED BURNING UPON BARK-BEETLE INFESTATIONS

A fire of an entirely different character was studied in Siskiyou County, Calif., where a large lumber company during the season of 1920 practiced on its own holdings what has been termed "controlled" or "light" burning. The method consisted of burning the area at night in small blocks of not more than 160 acres surrounded by fire lines. During the period from June 15, 1920, to about October 30, 1920, approximately 12,000 acres were burned over by this system. The object of this operation was to lessen the fire hazards within a tract where the company contemplated logging in the near future.

In character, the area differed essentially from those previously described in that there were few steep slopes, a very large part of the acreage being relatively flat. Because of this and the fact that the fires were controlled and the burning was carried on at night, there was very little evidence of severe damage to merchantable trees such as occurred on the burns described. The character of this fire also differed from the others in that the burning operations were extended over a considerable period of time.

Before the burning was started in 1920, representatives of the Bureau of Entomology made a 100 per cent cruise of one section of 640 acres, marking all insect-infested trees. This section, which was among the first to be covered by the burning operations, was burned over in July. One month later a second cruise was made of the same section.

These examinations yielded the following data:

	Number of trees
Trees infested at time of fire (first generation, 1920)	43
Brood mortality observed in infested trees exposed to fire	0
Number of standing trees attacked subsequent to fire	78
Number of trees burned down by fire in butt scars and subsequently attacked by beetles	12
Total loss on section during 1920 (year of fire)	133
Total loss on section during 1919 (year preceding fire)	79
Increase during 1920	54

It would appear from these figures that the fire caused some attraction which brought beetles into the area during the latter part of

the summer in which the fire occurred. However, no such concentration as that found on the other burns was observed. The failure of the beetles to concentrate on the light-burned area may have been due to the absence of trees sufficiently injured by the fire to attract the beetles, and also to the fact that for the remainder of the flight period of that season fires were running through near-by timber, creating conditions similar to those on the section that was cruised. This section was logged off the following spring, so that no follow-up studies were possible.

At the time that the burning operations were in progress the same company, under the technical advice of the Bureau of Entomology, organized a project for the control of the western pine beetle on a tract of 44,000 acres adjoining the 12,000 acres covered by controlled burning. From cruises made during the course of this project it was found that the loss during 1920 on the entire tract averaged 77 trees per section, or 112 board feet per acre, or 1.4 per cent of the total pine stand. Direct control measures against the beetles were started in the fall of 1920. The methods consisted of locating the infested trees, felling, and peeling and burning the infested bark. Two sections that had been burned over by the controlled burning of the summer extended into the area that was included in the insect-control work. As a 100-per cent cruise was made of these two sections and the adjoining area, it was possible to compare the overwintering infestation on the burned and unburned areas.

Number of infested trees per section on burned area	189
Number of infested trees per section on adjoining unburned area	170

It is evident that in this class of fire a very limited concentration of beetles resulted. Practically all of the area covered by the light-burning operations in 1920 was logged off during the following two seasons, so that it was possible to study only the results which developed immediately after the fire. As both control work and logging operations were carried on within the area subsequent to the burning, the natural tendencies of the infestation were considerably disturbed.

GENERAL SUMMARY

Forest fires of sufficient severity to scorch the bark, cambium, and foliage of yellow pine trees produce types of injury which make certain trees especially attractive to the western pine beetle. Many trees which have been only moderately injured by the fire and are apparently capable of recovering are attacked and killed by the beetles after a fire of this character.

The attraction of fire-injured trees often causes a concentration of beetles within a burned area which lasts for one or two seasons following the fire. This attraction may extend for a distance of 2 or 3 miles from the burn.

The concentration of bark-beetle attacks in fire-injured trees within a burned area does not develop into an epidemic condition. The loss from bark-beetle attacks in trees not injured by the fire either within the area of the burn or in the surrounding forest is not materially increased as a result of this concentration.

Fires which are not severe enough to burn the bark from infested trees are of little value in controlling bark-beetle infestations. Fires of moderate severity do not kill bark-beetle broods in infested trees that are on the area at the time of the fire.

While fire-injured trees attract bark beetles in considerable numbers they do not always afford favorable breeding conditions for the new broods. Where the trees have been defoliated by fire, an abnormally moist condition of the inner bark may follow, which results in high mortality of the broods in the early larval stages. Conditions have been observed where this mortality was so great as to act as a controlling influence, fewer beetles emerging from these trees than attacked them.

By and large, these studies show that fires can be of but little benefit in reducing beetle losses through killing the beetles unless the fires are severe enough to kill the trees; neither do fires have any permanent effect in increasing beetle losses in surrounding timber. The principal rôle of bark beetles in connection with forest fires is in the added destruction by the insects of moderately fire-injured trees, which otherwise would have survived.

Thus bark beetles supplement and increase the timber losses initiated by forest fires, while fires have but little influence in permanently increasing the losses caused by bark beetles.

46653—27——2

THE PARASITES OF THE PINE TIP MOTH, RHYACIONIA FRUSTRANA (COMSTOCK) [1]

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INTRODUCTION

The Nebraska National Forest is an entirely artificial plantation of pines of various species, situated in the sand hills just west of Halsey, Nebr. Unfortunately, with some of the young trees brought in for planting, certain pine-infesting insects were introduced into the forest. Among these was a tip moth, *Rhyacionia frustrana* var. *bushnelli* Busck, which has increased to such an extent that it has killed or badly stunted a very large percentage of the western yellow pines, the most favorable species for the development and increase of the pest.

In 1924 the Forest Service and the Bureau of Entomology instituted an attempt to control the insect by means of parasites.

To determine what eastern insects were available for introduction, the writer in 1924 made a preliminary study of the parasites of the eastern form of the tip moth, *Rhyacionia frustrana* (Comstock). Several thousand tips of *Pinus virginiana* infested by the summer generation of the tip moth were collected, principally in the vicinity of Falls Church, Va., and the insects contained in these were reared. A few parasitized tip worms were also collected in the spring of 1925. In the rearings were 21 species of parasitic Hymenoptera and two species of parasitic flies. A small lot of material collected at Bogalusa, La., by R. A. St. George, of the Bureau of Entomology, produced six species of parasitic Hymenoptera.

There had previously been recorded only three species of parasites of *Rhyacionia frustrana*. In 1890 Packard [2] reported as parasites a species of Bracon and a species of Perilampus, and in 1921 Cushman [3] listed this insect among the hosts of *Itoplectis conquisitor* (Say). In the meantime Carl Heinrich, then of the Division of Forest Insect Investigations, had reared some parasites from Virginia and Massachusetts; and a few other parasites of the species from other localities had been reared by members of the division.

All of these parasites are classified and discussed below on the basis of their relation to the host insect. A key for the identification of the parasites is appended.

[1] Received for publication Jan. 18, 1927; issued May, 1927.

[2] PACKARD, A. S. INSECTS INJURIOUS TO FOREST AND SHADE TREES. U. S. Ent. Comn. Rpt. 5: 751. 1890. (Rev. and enl. ed. U. S. Ent. Comn. Bul. 7, 1881.)

[3] CUSHMAN, R. A. THE NORTH AMERICAN ICHNEUMON-FLIES OF THE TRIBE EPHIALTINI. U. S. Natl. Mus. Proc. 58: 350. 1921.

EXTERNAL PARASITES OF LARVA

HYMENOPTERA

BETHYLIDAE

GONIOZUS LONGICEPS KIEFFER

Goniozus longiceps, originally described from Texas, has been reared in small numbers from tip-moth larvae from Bogalusa, La. It is probably gregarious on full-grown or nearly full-grown larvae.

ICHNEUMONIDAE

EPIURUS INDAGATOR (CRESSON)

Epiurus indagator is a common parasite of internally feeding microlepidopterous larvae, with many known hosts. It attacks full-grown or large larvae and does not confine its attack to insects infesting coniferous trees. Its distribution covers the eastern half of the United States and southern Canada. In the rearings of 1924, at Falls Church, it was rather rare. This species is solitary; the cocoon consists of a thin silken lining to the burrow of the host.

CALLIEPHIALTES COMSTOCKII (CRESSON)

Calliephialtes comstockii apparently confines its attack to lepidopterous larvae infesting coniferous trees, but prefers larger species than *frustrana*. It might do well on variety *bushnelli*, which is larger, but the rearings of 1924 at Falls Church on *frustrana* produced only a few undersized males. It is solitary on full-grown larvae. It spins a light brownish, semitransparent cocoon. Its distribution is about like that of Epiurus, except that it is confined to regions of coniferous forests.

BRACONIDAE

MICROBRACON MELLITOR (SAY)

This is a very common species, of catholic taste as regards hosts, attacking almost any internally feeding larva, whether lepidopterous or coleopterous. It is one of the principal parasites of the cotton boll weevil and of the pink bollworm. It is distributed throughout the eastern half of the country. In the 1924 Falls Church rearings from *frustrana* it was rare. It is solitary on full-grown or nearly full-grown larvae, and pupates in a white to brownish, opaque cocoon.

MICROBRACON GEMMAECOLA (CUSHMAN)

A few specimens of this species were reared at Falls Church in 1925 but none in 1924. In 1926 it was the most abundant parasite in Nantucket. It is very similar in its habits to *mellitor*.

MICROBRACON GELECHIAE (ASHMEAD)

Microbracon gelechiae is a gregarious parasite of internally feeding microlepidopterous larvae, from a few to several developing on a single host. It attacks large larvae. It occurs in the eastern half of the country and on the west coast. In the 1924 rearings at Falls Church it was very rare. It spins a dense brown cocoon.

EURYTOMIDAE

EURYTOMA TYLODERMATIS (ASHMEAD)

Eurytoma tylodermatis was the most abundant of all the parasites in the 1924 rearings at Falls Church. It is normally primary but occasionally secondary. It attacks large larvae, and is parasitic on many internally feeding lepidopterous and coleopterous larvae over at least the eastern half of the United States and southern Canada. The species is solitary.

EULOPHIDAE

SECODELLA SUBOPACA (GAHAN)

This is a solitary parasite of small larvae. There were a few females in the 1924 rearings at Falls Church and a single male from Bogalusa, La.

HYSSOPUS THYMUS (GIRAULT)

Hyssopus thymus is a minute gregarious parasite of full-grown or nearly full-grown larvae, from 3 to 10 or 12 developing on a single host. It was rather rare in the 1924 and 1925 rearings at Falls Church. It was originally described from Lincoln, Nebr.

HYSSOPUS RHYACIONIAE (GAHAN)

Hyssopus rhyacioniae is exactly similar in habit to *H. thymus*, but is less common. It was reared at Falls Church in 1924.

ELACHERTUS PINI GAHAN

This is another gregarious parasite, similar in its host relations to the species of Hyssopus, but less abundant. It was reared only at Falls Church.

PTEROMALIDAE

HABROCYTUS THYRIDOPTERIGIS HOWARD

Habrocytus thyridopterigis was reared sparingly at Falls Church in 1924 as a solitary primary parasite and in 1925 as a primary and in one case as a secondary parasite, the intermediate host being *Campoplex frustranae*, a cocoon of which produced five males of Habrocytus. In 1916 *H. thyridopterigis* was reared at Falls Church by Heinrich, and in that year it was apparently one of the most abundant parasites of the tip moth. Heretofore it has been recorded only as a secondary parasite of the bagworm, *Thyridopteryx ephemeraeformis* Haworth, and the white-marked tussock moth, *Hemerocampa leucostigma* Smith and Abbot.

INTERNAL PARASITES OF LARVA

HYMENOPTERA

ICHNEUMONIDAE

COMPOPLEX FRUSTRANAE CUSHMAN

Next to *Eurytoma tylodermatis*, *Campoplex frustranae* was the most abundant parasite in the 1924 rearings at Falls Church. It attacks its host probably when the latter is comparatively young, and spins its white cocoon within the shattered shell of the newly formed pupa of the host.

CREMASTUS EPAGOGES CUSHMAN

This species was of rare occurrence in the 1924 rearings at Falls Church. In habit it is similar to Campoplex, but leaves its host while the latter is still in the larval stage and spins a dense dark-brown cocoon. It was originally described from specimens reared at Nashville, Tenn., from *Epagoge sulfureana* Clemens.

GLYPTA VARIPES CRESSON

Glypta varipes was reared from the tip moth only at Sharon Heights, Mass., in 1916, and at Nantucket, Mass., in 1926, where it appears to have been one of the more important parasites. This species is solitary.

BRACONIDAE

PHANEROTOMA RHYACIONIAE CUSHMAN

Phanerotoma rhyacioniae is known only from the tip moth and only from Bogalusa, La., where, judging from the small amount of material available, it was apparently the most important parasite. No observations on its life history have been reported, but probably it deposits its egg in the egg of the tip moth, its larva leaving the host when the latter is full-grown and pupating in a cocoon in the burrow of the host. It is probably solitary.

APANTELES SPECIES

There was a single specimen of Apanteles from Bogalusa, La.; it probably attacks the very young larva, leaving its host when the latter is nearly full-grown.

DIPTERA

TACHINIDAE

LIXOPHAGA MEDIOCRIS ALDRICH

The third most abundant parasite in the 1924 rearings at Falls Church was *Lixophaga mediocris*. The full-grown larva emerges from the larva of the host and pupates in the host burrow. It was described as a new species from these specimens.

LIXOPHAGA PLUMBEA ALDRICH

This is another new species with habits exactly similar to those of *Lixophaga mediocris*. It was much less abundant than the latter species at Falls Church in 1924.

PARASITES OF PUPA

HYMENOPTERA

ICHNEUMONIDAE

ITOPLECTIS CONQUISITOR (SAY)

This is one of the most common Ichneumonidae throughout the eastern half of the United States, southern Canada, and the West Indies, parasitizing lepidopterous pupae of nearly all groups. It is rare as a parasite of the tip moth, which is too small to be a favorable host; only a few undersized males were reared. It pupates within the host pupa.

CHALCIDIDAE

HALTICHELLA RHYACIONIAE JAHAN

Of all the parasites in the 1924 rearings at Falls Church, *Haltichella rhyacioniae* ranked fourth in point of abundance, and it was also one of the most abundant in Heinrich's 1916 rearings. There was one specimen from Nantucket, Mass. This species pupates in the host pupa.

SECONDARY PARASITES AND THOSE OF UNKNOWN STATUS

Under this head are placed seven species of chalcidoids and one braconid, all reared in small numbers, on which no definite observations for determining their status were made. The first three, because of the known habits of their congeners, are almost certainly secondaries.

HYMENOPTERA

CHALCIDIDAE

SPILOCHALCIS DELIRA (CRESSON)

SPILOCHALCIS SPECIES

These are secondarily parasitic through pupal parasites.

PERILAMPIDAE

PERILAMPUS SPECIES

EUPELMIDAE

EUPELMUS CYANICEPS ASHMEAD

Eupelmus cyaniceps is a ubiquitous species of very wide distribution and catholic taste in the matter of hosts. It attacks Lepidoptera, Coleoptera, and Hymenoptera, sometimes as a primary parasite and sometimes as a secondary parasite. Its exact relationship to the tip moth is unknown, but very likely it is both a primary and a secondary parasite.

EULOPHIDAE

EPITETRASTICHUS CUNEIFORMIS GIRAULT

This species was reared in small numbers at Falls Church in 1924 and by Heinrich in 1916. It is probably not associated in any way with the tip moth, but rather with some species of midge infesting the tips. It was originally reared in such an association.

TETRASTICHUS SPECIES

There were two specimens from the Bogalusa, La., material.

ELASMIDAE

ELASMUS SETOSISCUTELLATUS CRAWFORD

One specimen of this species was reared at Falls Church in 1924, probably from a midge, such an insect having been the host in a previous rearing.

BRACONIDAE

HETEROSPILUS SPECIES

A single specimen of Heterospilus was reared from infested tips at Nantucket, Mass. Most likely Heterospilus is not parasitic on the tip moth, but in this instance was associated with some coleopterous borer.

HOW TO RECOGNIZE THE PARASITES

The following key will serve to identify the parasites listed above:

1. With one pair of wings and having the general appearance of a small house fly (Diptera)—*Lixophaga mediocris* Aldrich and *Lixophaga plumbea* Aldrich. (These two flies are very similar in appearance and distinguishable only to the trained eye.)
   With two pairs of wings and more wasplike in appearance, some very minute (Hymenoptera)—2
2. Wings with many veins (fig. 1, *a–e*)—3
   Wings with veins only along front margin (Chalcidoidea) (fig. 1, *f*)—15
3. Antennae very short and placed close to mouth; eyes very small (fig. 1, *h*); wings as in figure 1, *a* (Bethylidae)—*Goniozus longiceps* Kieffer
   Antennae long and slender and placed far above mouth; eyes large (fig. 1, *g*, *i*)—4
4. Front wing about as in Figure 1, *b* or *c* (Ichneumonidae)—5
   Front wing about as in Figure 1, *d* or *e* (Braconidae)—10
5. Abdomen flattened below, rounded above (fig. 1, *j*), the first segment broad at the base—6
   Abdomen compressed from side to side, the first segment long and very narrow at base (fig. 1, *k*)—9
6. Abdomen with segments edged above with whitish or yellowish—*Itoplectis conquisitor* (Say)
   Abdomen with segments entirely black above—7
7. Middle segments of abdomen with deep oblique furrows extending from middle of base toward hind angles (fig. 1, *j*)—*Glypta varipes* Cresson
   Middle segments of abdomen without oblique furrows—8
8. Hind tibia with alternate rings of white and black—*Epiurus indagator* (Cresson)
   Hind tibia white only at base—*Calliephialtes comstockii* (Cresson)
9. Head entirely black; front wing with the small cell marked *a* in Figure 1, *b*—*Campoplex frustranae* Cushman
   Head with yellow rings surrounding the eyes; front wing as in Figure 1, *c*, without cell *a*—*Cremastus epagoges* Cushman

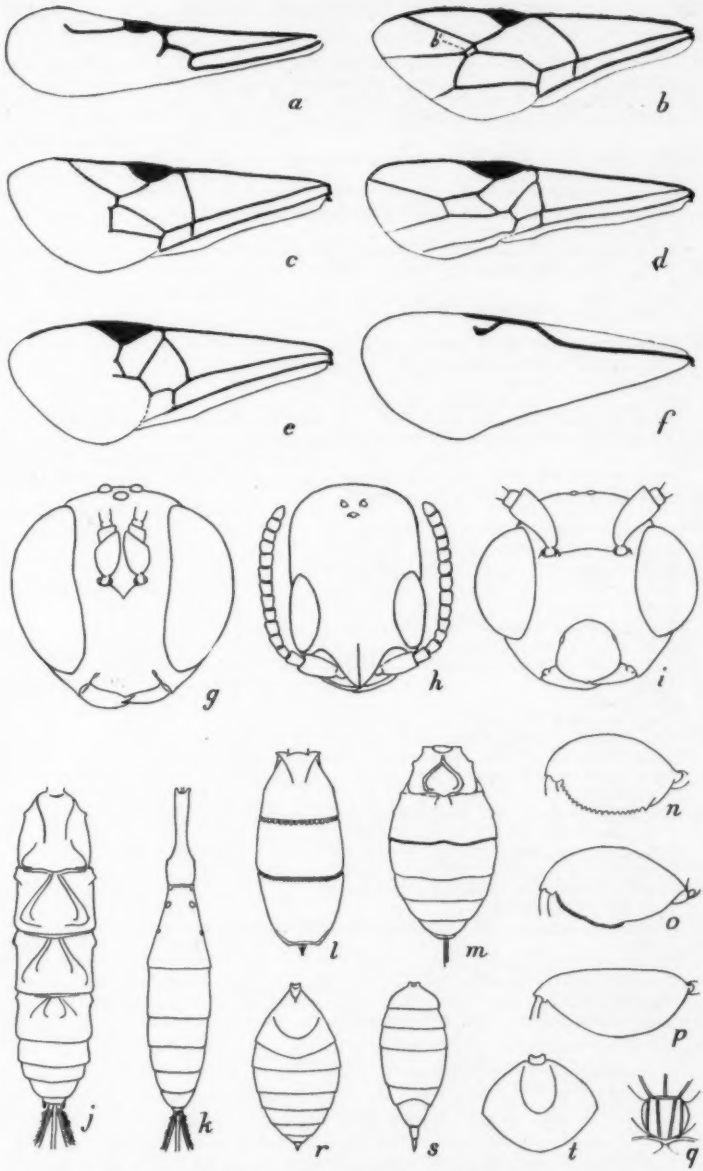

FIG. 1.—*a*, Forewing of *Goniozus longiceps* Kieffer; *b*, forewing of *Epiurus indagator* (Cresson); *c*, forewing of *Cremastus epagoges* Cushman; *d*, forewing of *Microbracon gemmaecola* Cushman; *e*, forewing of *Apanteles* species; *f*, forewing of *Eurytoma tylodermatis* Ashmead; *g*, head of *Campoplex frustranae* Cushman; *h*, head of *Goniozus longiceps* Kieffer; *i*, head of *Phanerotoma rhyacioniae* Cushman; *j*, abdomen of *Glypta varipes* Cresson; *k*, abdomen of *Campoplex frustranae* Cushman; *l*, abdomen of *Phanerotoma rhyacioniae* Cushman; *m*, abdomen of *Microbracon gemmaecola* Cushman; *n*, hind femur of *Spilochalcis delira* (Cresson); *o*, hind femur of *Haltichella rhyacioniae* Gahan; *p*, hind femur of *Elasmus setosiscutellatus* Crawford; *q*, scutellum of *Epitetrastichus cuneiformis* Girault; *r*, abdomen of *Hyssopus thymus* Girault; *s*, abdomen of *Eurytoma tylodermatis* Ashmead; *t*, abdomen of *Perilampus* species

10. Wings as in Figure 1, *e*; body entirely black ..... *Apanteles* sp.
    Wings more similar to Figure 1, *d*; body usually more or less yellowish or reddish 11
11. Abdomen with only three visible segments (fig. 1, *l*) ..... *Phanerotoma rhyacioniae* Cushman
    Abdomen with more than three visible segments (fig. 1, *m*) 12
12. Largely blackish ..... *Microbracon gelechiae* Ashmead
    Largely reddish yellow 13
13. First segment of abdomen with a triangular area set off by deep grooves as in Figure 1, *m* 14
    First segment of abdomen without such an area ..... *Heterospilus* sp.
14. Wings distinctly smoky; ovipositor fully as long as abdomen ..... *Microbracon mellitor* (Say)
    Wings clear; ovipositor shorter than abdomen ..... *Microbracon gemmaecola* Cushman
15. Hind femur very deep (fig. 1, *n–p*) 16
    Hind femur not especially deep 19
16. Body entirely black 17
    Body more or less spotted with yellow 18
17. Body and legs strongly flattened from side to side and having somewhat the appearance of a winged flea; minute ..... *Elasmus setosiscutellatus* Crawford
    Body stout, not flattened from side to side ..... *Haltichella rhyacioniae* Gahan
18. Abdomen largely reddish with several yellow spots ..... *Spilochalcis delira* (Cresson)
    Abdomen black with two yellow spots ..... *Spilochalcis* sp.
19. Bright metallic green, blue, or bronze 20
    Black with at most faint metallic luster 22
20. Blue; very minute ..... *Secodella subopaca* Gahan
    Green or bronze 21
21. Body entirely bright green; ovipositor (the sting) not protruding ..... *Habrocytus thyridopterigis* Howard
    Not entirely bright green; ovipositor protruding and ringed with yellow ..... *Eupelmus cyaniceps* (Ashmead) (female)
22. Dark blue ..... *Eupelmus cyaniceps* (Ashmead) (male)
    Black or black and brown 23
23. Scutellum with four longitudinal grooves (fig. 1, *q*) 24
    Scutellum with at most two longitudinal grooves 25
24. Head more or less brownish ..... *Epitetrastichus cuneiformis* Girault
    Head black ..... *Tetrastichus* sp.
25. Abdomen very broadly oval, much broader than deep (fig. 1, *r*.; very minute 26
    Abdomen not broadly oval above, lanceolate or broadly triangular (fig. 1, *s*, *t*) 28
26. Abdomen with a whitish spot at base ..... *Elachertus pini* Gahan
    Abdomen entirely black 27
27. Thorax prolonged in front so that the head appears to be set out on a neck ..... *Hyssopus rhyacioniae* Gahan
    Thorax not prolonged in front ..... *Hyssopus thymus* Girault
28. Abdomen flattened from side to side (fig. 1, *s*); joints of antennae in male beadlike, with long hair ..... *Eurytoma tylodermatis* Ashmead
    Abdomen triangular (fig. 1, *t*); antennae in male not as above ..... *Perilampus* sp.

ADAPTATION OF THE BACTERICIDAL ACTION OF CHLOROFORM TO THE PREPARATION OF BACTERINS [1]

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REVIEW OF RELATED WORK

It is not the purpose of this paper to herald a discovery, but rather to call attention to an unusual, but practical, application of facts already at hand relating to the killing of vegetative bacteria by exposing them to bactericidal vapors, especially to that of chloroform. (*3, p. 166.*)[2]

Chloroform has long been widely accepted as a preservative. When it is added to immune sera (*5, p. 667*), the reason is obviously to check contaminating growth, rather than to "kill off" a specific organism in the fluid, as in the case of bacterins.

It has long been known (*1, p. 117*) that, although chloroform can inhibit the activity of microorganisms, it has no effect on their enzymes. On the other hand, the destructive effect of even moderate heat upon the products of bacteria has been generally emphasized. Hiss and Zinsser (*2, p. 43, 168*) assert that—

> temperatures of over 70° C. permanently destroy most enzymes. * * * Against the weaker disinfectants in common use, enzymes often show a higher resistance than do the bacteria which give rise to them. * * * Both of these substances [chloroform and toluol] will destroy the bacteria without injuring the enzymes.

In discussing the preparation of vaccines, Kolmer (*4, p. 657*) says:

> The specific microorganism or virus used in a vaccine should be modified as little as possible, or just sufficient to rob it of its disease-producing power. * * * The less modification the better the vaccine. If the exposure [to heat] is too prolonged or the temperature too high, the vaccinogenic power of the bacilli is destroyed, and the suspension in salt solution is no more potent or of no greater value than the salt solution itself. Therefore the nearer the vaccine approaches the fully viable virus or microorganism, the more potent it will be.

The known destructive action of heat upon the products of bacteria as compared with the lack of such action exerted upon these products by chloroform would appear, in the light of Kolmer's statement, to be a fair criterion of the conversely relative degrees of potency of bacterins made by these respective methods. Unpublished data, accumulated by the writer in connection with extensive experiments on the biological therapy of bovine mammitis, have shown clearly the greater potency of autogenous bacterins treated with chloroform as compared with those treated with heat.

These findings are in harmony with those of Vincent and Collignon (*6*), who observed that certain bacterial suspensions could be killed

[1] Received for publication Jan. 4, 1927; issued May, 1927.

[2] Reference is made by number (italic) to "Literature cited," p. 630.

by direct exposure to ether, and could be employed advantageously for immunization. This method is discussed by them in part as follows:

> The antigen employed was prepared according to the principle which one of us had made known for the preparation of antityphoid serum. The cultures of the micrococcus (Binot), three days old on gelatin, were emulsified in physiological salt solution (10 c. c. for a large tube of culture), after which ether was added. The mixture, vigorously shaken for one or two minutes, was carefully corked up, and then left for 24 hours at laboratory temperature. After this interval the ether was evaporated out at a temperature of 38° for a few minutes. This culture, thus killed, was employed as a vaccine * * *.
>
> It was hoped that we might be able to obtain a practical immunization of the goat against Malta fever by several subcutaneous injections of cultures of the microbe of that disease, sterilized by ether, which was a volatile antiseptic, easily manipulated and easily disposed of. Thus treated, the virus lost none of its immunizing properties and promoted the production of antibodies almost as energetically as would a living culture. For the purpose of immunizing animals, this antigen is far preferable to a living attenuated culture of the *M. melitensis*, because the living culture, even though it is attenuated, might cause the animals to become disseminators of the germ. It was equally superior to cultures killed by heat.

TESTS WITH CHLOROFORM—SUSPENSION METHOD

In the laboratory of the Pathological Division the need of an effective method of sterilizing bacterial suspensions without heat was recognized. In some instances the use of serum broth for the propagation of the bacteria was attended with good results until the process reached the stage of sterilization by heat. During this operation, however, the serum in the medium became coagulated and clouded the product, to its obvious detriment.

Out of this exigency was conceived the idea of sterilization by other means than heat. The use of certain chemicals offered a possible solution of the problem. Chloroform was first employed in the following manner: Beef-infusion broth, containing about 1 per cent of normal beef serum, was sterilized by passage through a bacteria-retaining filter. This broth was distributed into sterile 200 c. c. Erlenmeyer flasks, 100 c. c. of broth to each flask. Sterility was tested and proved by inoculation.

Six of these flasks were then inoculated with a culture of *Staphylococcus aureus* and incubated 24 hours. Test cultures were made on agar slants and heavy growths resulted. Meanwhile the cotton plugs were removed from the flasks, rubber stoppers were substituted, and a small homeopathic vial was suspended by a slender wire in each flask (fig. 1). One cubic centimeter of chloroform was placed in each vial immediately before the vials were inserted in the flasks. The flasks were left at room temperature, which was then noted to be 79° F. Variation from this temperature was so slight as to be negligible during the course of the experiment.

At the expiration of four hours after the suspension of the chloroform vials within the flasks test cultures were made on agar from each flask, and all were found to contain the living organism. Again after the expiration of 24 hours following the suspension of the vials, a second set of tests of cultures was made. Twenty-four hours' incubation proved them all to be sterile. At this time, exactly 48 hours after the commencement of the experiment, it was observed that the chloroform had entirely evaporated from within the vials. The

vials were then aseptically removed, and the stoppers replaced. The flasks were left at room temperature for a period of six days and three hours, after which cultures were made in agar to confirm the sterility of the suspensions. During the six-day period the rubber stopper had inadvertently become dislodged from flask No. 5, and the broth in this flask had become contaminated. With this exception, all the flasks of broth culture proved to be still sterile.

To test quantitatively the amount of chloroform required to kill 100 c. c. of the serum-broth culture, a second series of tests was made. Six flasks were inoculated with *Pseudomonas pyocyanea*, and incubated. Test cultures showed the presence of living organisms in all flasks.

After five days the plugs were aseptically removed, as before, and the rubber stoppers and suspended vials containing chloroform were substituted. Different amounts of chloroform were employed, as follows: Flask No. 1, 0.3 c. c.; No. 2, 0.5 c. c.; No. 3, 1.0 c. c.; No. 4, 3.0 c. c.; No. 5, 5.0 c. c.; and No. 6, viability control, none.

After a lapse of 2 hours and 50 minutes, and again of 4 hours and 30 minutes, test cultures from all six flasks were made on agar, which proved by incubation to contain the organism in every instance.

After a total lapse of 21½ hours another set of test cultures was made. All proved to be sterile except that in flask No. 6. This flask had received no chloroform fumigation, and now contained a heavy suspension of viable organisms.

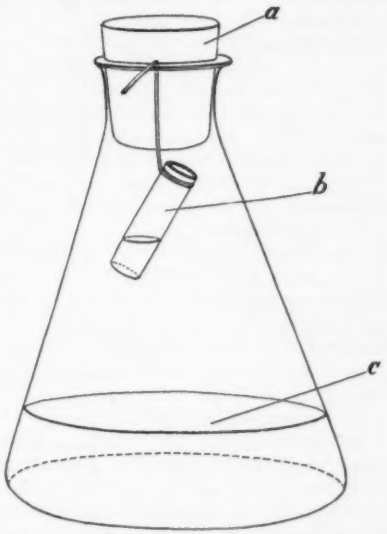

FIG. 1.—Apparatus for sterilizing cultures with vial containing chloroform: *a*, Rubber stopper; *b*, small vial suspended from stopper, containing chloroform; *c*, broth culture of living organisms

At this time observation was taken on the quantity of chloroform remaining in the vials. The observations, roughly tabulated, are as follows: Flask No. 1, chloroform completely evaporated; No. 2, completely evaporated; No. 3, nearly all evaporated; No. 4, nearly all present; No. 5, nearly all present.

It was thus demonstrated that as low a proportion as 0.3 per cent of chloroform would sterilize a suspension of *Pseudomonas pyocyanea* in 21½ hours at incubator temperature.

POTENCY TESTS OF CHLOROFORM AND OTHER CHEMICALS

An experiment was instituted to test the relative bactericidal potency of several volatile chemicals in different proportions, against a nonspore-bearing aerobe.

With *Pasteurella boviseptica*, eight flasks of broth (100 c. c. each) were inoculated March 14 at 4 p. m. and placed in the incubator.

On March 20 at 10 a. m. they were removed from the incubator, and at 3 p. m. chemicals were suspended in flasks as follows:

Flask No. 1, chloroform, 1 c. c.; flask No. 2, chloroform, 0.1 c. c.; flask No. 3, formaldehyde solution United States Pharmacopoeia, 1 c. c.; flask No. 4, formaldehyde solution, United States Pharmacopoeia, 0.1 c. c.; flask No. 5, ammonia fortior, 1 c. c.; flask No. 6, ether, 1 c. c.; flask No. 7, ether, 0.1 c. c.; flask No. 8, control—no chemical.

Test agar-slant cultures (a) made at this time proved to contain living *Pasteurella boviseptica* organisms. No. 5, however, was contaminated.

On March 21 at 11 a. m. (20 hours later), test agar-slant cultures (b) were made from each flask, which upon incubation gave the following results:

Culture No. 1–b, sterile; culture No. 2–b, growth of *Pasteurella boviseptica* plus contaminating mold; culture No. 3–b, growth of *P. boviseptica;* culture No. 4–b, growth of *P. boviseptica;* culture No. 5–b, growth of *P. boviseptica;* culture No. 6–b, growth of *P. boviseptica;* culture No. 7–b, growth of *P. boviseptica;* culture No. 8–b, growth of *P. boviseptica.*

On March 23 at 11 a. m. (68 hours after beginning fumigation) test agar-slant cultures (c) were made from each flask, with the following results:

Culture No. 1–c, sterile; culture No. 2–c, growth of *Pasteurella boviseptica;* culture No. 3–c, sterile; culture No. 4–c, sterile; culture No. 5–c, growth of *P. boviseptica;* culture No. 6–c, sterile; culture No. 7–c, growth of *P. boviseptica;* culture No. 8–c, growth of *P. boviseptica* (control).

It is apparent that formaldehyde solution U. S. P. kills in the same and even in smaller quantities than chloroform, but the latter is more desirable in that its action is more rapid. This may be attributable to the fact that chloroform vapor, being heavier than air, concentrates more fully upon the exposed surface of the culture. Ether vapor, although effective in its bactericidal action, is undesirable for practical use because of its high inflammability.

CHLOROFORM TESTS WITH VARIOUS ORGANISMS

At the conclusion of these experiments various organisms were tested for susceptibility to sterilization by chloroform fumigation such as had proved effective for *Staphylococcus aureus, Pseudomonas pyocyanea,* and *Pasteurella boviseptica.*

The following nonspore-bearing organisms were implanted each in a 200 c. c. flask, containing 100 c. c. of plain infusion boullion: *Aerobacter cloacae, Alcaligines abortus, A. bronchisepticus, A. melitensis, Chromobacterium violaceum, Corynebacterium ovis, Eberthella sanguinarium, Erysipelothrix rhusiopathiae, Erythrobacillus prodigiosus, Escherichia coli* (swine origin), *Hemophilus pyogenes, Pasteurella boviseptica, Salmonella abortivo-equina, S. aertrycke, S. paratyphi, S. pullorum, S. suipestifer, Staphylococcus aureus, S. citreus,* Streptococcus (canine).

These cultures were placed in an incubator and removed after 46 hours' incubation. Control cultures (series A) were made and 1 c. c. of chloroform was then suspended in each flask in the manner

previously described. All the series A control cultures had a good growth after 24 hours' incubation except *Hemophilus pyogenes*, *Alcaligines abortus*, and *A. melitensis*. Since these are very delicate and slow-growing organisms, they were continued in the experiment, as it was thought that longer incubation might establish the viability of the cultures. After an additional 24 hours' incubation at room temperature an appreciable growth of *A. abortus* and *A. melitensis* was noted, but the culture of *H. pyogenes* remained sterile, and was therefore eliminated as having been a dead culture from the beginning of the experiment.

Control cultures (series B) were made after the broth cultures had been subjected to chloroform fumigation for 24 hours. At this time the vials were removed and the rubber stoppers replaced.

After 24 hours' incubation these controls were found to be sterile except for a few colonies on culture tube No. 15 (Streptococcus [canine]) and tube No. 37 (*Aerobacter cloacae*). It was thought possible that the stoppers had been left sufficiently loose to permit the escape of the chloroform fumes before their bactericidal action had taken place. New flasks of broth were therefore inoculated with each of these organisms and this part of the experiment repeated. A tube of serum agar was also inoculated from each of the original flasks.

After four hours' incubation, test cultures of these two organisms (series A) were made from each flask on to serum agar, and then 1 c. c. of chloroform was suspended in each flask, rubber stoppers inserted, and all were left at room temperature. Upon incubation, a luxuriant growth was obtained of all test cultures (series A).

The chloroform vials were removed after 24 hours and test cultures (series B) were made on agar slants. These were incubated for 24 hours, at the end of which time they were all found to contain typical growths. It was concluded, therefore, that this method of sterilization was not effective in killing very resistant nonspore-bearing aerobes. It should be added, however, that the writer has repeatedly employed this method for killing streptococci of diverse origin, with entirely satisfactory results, indicating that the streptococcus employed as culture No. 15 must have possessed extraordinary qualities of resistance.

Three spore-bearing aerobes were planted in separate flasks of 100 c. c. of broth each, preparatory to determining what action, if any, chloroform fumigation would exert on this class of bacteria. The organisms used were *Bacillus megatherium*, *B. X*, and *B. vulgatus*. After 20 hours, test agar cultures (series A) were made from each flask, and 1 c. c. of chloroform was then suspended in each flask. Rubber stoppers were inserted instead of the cotton plugs, and all flasks were left at room temperature. All the test cultures proved to contain the viable organisms.

After 24 hours the chloroform vials were removed and test cultures series B) were made on agar slants. These were incubated for 24 hours, after which they were found to contain luxuriant growths of the organisms. It was, therefore, obvious that chloroform fumigation as here practiced would not be applicable to the sterilization of cultures of these spore-bearing aerobes.

TESTS WITH CHLOROFORM VAPOR

At the suggestion of John S. Buckley, chief of the Pathological Division, the writer directed his attention to the possibility of accomplishing the sterilization of fluid suspensions of organisms by the passage of chloroform vapor through them by means of air pressure or vacuum.

For this purpose an apparatus was developed, as illustrated in Figure 2, consisting of an Erlenmeyer flask, *a*, and a graduated

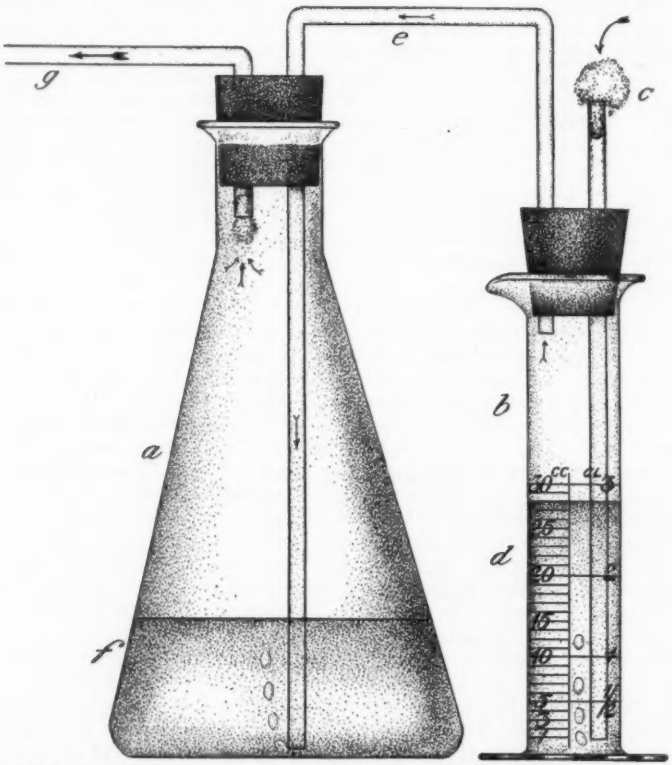

FIG. 2.—Apparatus for sterilizing with chloroform vapor: *a*, Erlenmeyer flask; *b*, graduated cylinder; *c*, inlet tube; *d*, column of chloroform; *e*, connecting tube; *f*, broth culture; *g*, vacuum connection

cylinder, *b*, both equipped with rubber stoppers fitted with an inlet and an outlet glass tube. Tube *c*, the inlet tube for the graduated cylinder, has inserted in its upper extremity a loose plug of cotton wool for the filtration of the air. Passing through this tube, the filtered air is drawn through a column of chloroform, *d*, of known volume, and then the air, now surcharged with the chloroform vapor, is carried through the bent, connecting tube *e* (which comprises the outlet tube of the graduated cylinder and the inlet tube of the Erlenmeyer flask) from the air space of the graduated cylinder, *b*,

to the depths of the broth culture, *f*. As the chloroform vapor passes upward through the broth culture, some part of it becomes absorbed in the medium, and the residue, together with the air, escapes through the vacuum connection, *g*, which is fitted with a cotton plug within the flask, *a*.

The following experiments in sterilization with streaming chloroform vapor were performed at room temperature. A series of agar cultures (series A) was made from three 500 c. c. flasks, each containing 100 c. c. of a broth culture of *Staphylococcus aureus*. These agar cultures developed luxuriant growths of the organism.

By the use of the apparatus just described, flask No. 1, containing 100 c. c. of the *Staphylococcus aureus* broth culture, was subjected to a current of streaming air charged with the vapor of chloroform. The amount of vacuum employed was not measured, but was such as to set up a lively ebullition in the chloroform, and also in the culture flask. Care was taken, however, that this bubbling activity should not become so violent as to carry over drops of liquid chloroform into the culture. After this process had continued for 10 minutes the column of chloroform was measured, and it was observed by comparison with the original measure of the column that 2.5 c. c. of chloroform had been vaporized and passed through the broth culture. This culture was then seeded upon agar (series B) and this, when incubated, yielded a heavy growth of *S. aureus*.

A similar process was carried out with a second flask (No. 2), containing a like culture. This time, however, the ebullition was less violent and was continued for 30 minutes. The quantity of chloroform converted into vapor and passed in this condition through the culture was 5 c. c. Series B cultures from this flask likewise yielded a heavy growth of the organism.

At the end of 24 hours a third set of agar cultures (series C) was made from flasks Nos. 1 and 2, and these, upon incubation, showed only a sparse, scattered development of colonies, indicating that a decided bactericidal action had taken place in both flasks of broth culture after the previous test cultures had been made. This bactericidal action was obviously due to the amount of chloroform which had been held in solution by the broth cultures. The prolonged preservative effect of such retained chloroform renders it unnecessary to add phenol as a preservative to cultures promptly sealed after being killed with chloroform.

A third test was carried out under conditions similar to those in the previous tests except that by the reduction of the vacuum to a minimum the period of ebullition was increased to 2 hours and 45 minutes, and the quantity of chloroform used was 4.5 c. c. The series B cultures from the third test proved to be sterile.

The advantage of this method over the chloroform-suspension method described earlier in this paper is that it accomplished the sterilization of the culture in a few hours instead of a day. Thus, in the routine employment of this method, practically a day is gained in the time required to prepare a bacterin for use. In the preparation of autogenous bacterins it is quite conceivable that this saving of a day might be a matter of the utmost importance to the patient.

It might be added that the quantity of chloroform retained in suspension in the bacterin is inconsequential so far as any detrimental

effects upon the patient is concerned. In the numerous injections of chloroform-killed suspensions into cattle and poultry performed by the writer, or under his direction, not the slightest local or systemic effect of chloroform has ever been noted.

SUMMARY AND CONCLUSIONS

Heat is destructive to the products of bacteria. The use of heat in the preparation of bacterins therefore reduces their potency.

Chloroform kills many nonspore-bearing bacteria with no important modification of their biochemical characteristics. The use of chloroform in the preparation of bacterins therefore conserves their potency. The preparation of killed cultures of spore-bearing organisms, and of nonspore-bearing organisms of resistant types is, however, not practicable by this method.

Chemicals such as formaldehyde solution U. S. P., ether, and possibly toluol, might be employed in the preparation of bacterins, but all are open to some objections.

The passage of 2.5 per cent of choloroform in streaming vapor through a broth culture of *Staphylococcus aureus* will not sterilize it in 10 minutes.

The passage of 5.0 per cent of choloroform in streaming vapor through a broth culture of *Staphylococcus aureus* will not sterilize it in 30 minutes.

The passage of 4.5 per cent of choloroform in streaming vapor through a broth culture of *Staphylococcus aureus* will sterilize the culture, provided the process is so retarded as to utilize that amount of chloroform, in a period of 2 hours and 45 minutes. It is important not only to pass an adequate quantity of vapor through the culture, but to retard the passage sufficiently to permit of maximum absorption of the vapor by the medium.

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THE NATURAL REPLACEMENT OF BLIGHT-KILLED CHESTNUT IN THE HARDWOOD FORESTS OF THE NORTHEAST [1]

By C. F. KORSTIAN, *Associate Silviculturist, Appalachian Forest Experiment Station,* and PAUL W. STICKEL, *Assistant Silviculturist, Northeastern Forest Experiment Station, Forest Service, United States Department of Agriculture* [2]

INTRODUCTION

Chestnut (*Castanea dentata*) once formed almost half the second-growth sprout hardwood forests of southern New England, New Jersey, and central and southern Pennsylvania; on small areas it often formed 90 per cent of the total wood volume. Its destruction by the chestnut blight (*Endothia parasitica*) has radically changed the composition of these forests. The rapid growth, abundance, and prodigious sprouting capacity of chestnut, together with its intrinsic value for a wide variety of uses, made it the most promising of the northeastern forest trees until the rapid spread of the blight made its extinction inevitable (*12*).[3]

The total loss of such a species naturally introduces serious problems as to the future silvicultural management of the forests of which it was an important component. These problems apply both to the North, where the chestnut is already dead, and to those parts of the Southern Appalachian region where chestnut still furnishes abundant and valuable forest products, but where it is already doomed (*4*, *5*, *13*).

Knowledge of what is happening on blight-killed chestnut areas is necessary in order to appraise the future growth rate of the changed forests and to determine the period of restoration of these lands to their full productive capacity with species other than chestnut.

Studies of the effects of the blight upon forests containing chestnut and upon the character and amount of natural replacement were undertaken as a first step toward supplying such information.

RESULTS OF 1920 STUDY OF CHESTNUT REPLACEMENT

The first field study of the natural replacement of chestnut by other species was made by the Forest Service, United States Department of Agriculture, in 1920, in western Virginia, Maryland, eastern Pennsylvania, and northern New Jersey.[4] Advance growth of desirable species sufficient to restock the stands was generally found. Larger-sized reproduction was not always present—thereby sometimes delaying future merchantable stands—although there were no vacant areas of any appreciable extent after the chestnut died. In some

[1] Received for publication Nov. 12, 1926; issued May, 1927.
[2] The writers wish to acknowledge the effective cooperation received during the progress of this study from Prof. R. C. Hawley, of the School of Forestry, Yale University; W. O. Filley, Forester, Connecticut Agricultural Experiment Station; W. M. Baker, Associate State Forester of New Jersey; and J. S. Illick, Chief of Information, Pennsylvania Department of Forests and Waters.
[3] Reference is made by number (italic) to "Literature cited," p. 648.
[4] HODSON, E. R. PRELIMINARY REPORT ON THE SILVICULTURE OF REPLACING THE CHESTNUT ON BLIGHT-KILLED AREAS. [Unpublished manuscript.]

stands, especially on the more favorable sites, advance reproduction was inadequate to restock the area at once. This, together with the presence of dense underbrush, suggested the possibility that the regeneration of desirable species might be long delayed, even if the value of the future forest were not permanently impaired.

OBJECT OF 1924 STUDIES

The 1920 study was followed in 1924 by somewhat similar but more intensive field studies of blight-depleted stands in the regions of earlier infection in southern New England and New Jersey. In addition, limited studies were made in Pennsylvania of the progress of replacement. The 1924 studies were extended to cover representative stands of different ages on the more important chestnut sites.

The primary object of these studies was to determine the progress of the natural replacement of the chestnut by other species, and to forecast as nearly as possible the impending changes in the composition of the stand.

In these studies the stands were examined for restocking and were then analyzed to determine the extent to which the replacing species are silviculturally desirable and economically valuable. These species were accordingly arranged in three classes.

Class 1—Desirable species: Red oak (*Quercus borealis*); white oak, (*Quercus alba*); black oak (*Quercus velutina*); chestnut oak (*Quercus montana*); hickory (*Hicoria* spp.); white ash (*Fraxinus americana*); sugar maple (*Acer saccharum*); sweet birch (*Betula lenta*); black cherry (*Prunus serotina*); yellow poplar (*Liriodendron tulipifera*); basswood (*Tilia glabra*); northern white pine (*Pinus strobus*); pitch pine [5] (*Pinus rigida*).

Class 2—Less desirable species: Scarlet oak (*Quercus coccinea*); red maple (*Acer rubrum*); beech (*Fagus grandifolia*); black gum (*Nyssa sylvatica*); aspen (*Populus tremuloides*); largetooth aspen (*Populus grandidentata*); eastern cottonwood (*Populus deltoides*).

Class 3—Undesirable species:[6] Dogwood (*Cornus* spp.); gray birch (*Betula populifolia*); bear or scrub oak (*Quercus ilicifolia*); sassafras (*Sassafras variifolium*); blue beech (*Carpinus caroliniana*); hop hornbeam (*Ostrya virginiana*); witch-hazel (*Hamamelis virginiana*); service berry (*Amelanchier canadensis*).

REPLACEMENT IN SOUTHERN NEW ENGLAND

Three areas were selected in Connecticut as representative of southern New England:

1. The Maltby tract of the New Haven Water Co., west of New Haven. (Figs. 1 and 2, A.) This tract has been under management since 1907, and has been described by Hawley (*8*, *9*).

2. The Meshomasick State Forest, north of Portland. Conditions in this forest have been described by Filley and Moss (*2*).

3. The Whittemore estate, adjacent to Lake Quassapaug, east of Woodbury—studied by Frothingham in 1910 (*3*).

Hawes (*7*) and Frothingham (*3*) have also described conditions in the second-growth forests of Connecticut before the chestnut was killed by the blight.

[5] In Pennsylvania and New Jersey pitch pine is a class 1 species, but in New England it is very seldom associated with chestnut.

[6] Various other shrubs large enough to appear in the records were also included in this class.

STUDIES ON PERMANENT SAMPLE PLOTS

The Connecticut areas were selected for study largely because they contained permanent sample plots which were established in other studies. Records of some of these plots are available as far

FIG. 1.—A well-managed stand of second-growth hardwoods in southern Connecticut. The chestnut has been salvaged and the stand, now composed chiefly of oaks, has fully recovered and still contains ample advance reproduction

back as 1904, the year that the blight was first discovered in Bronx Park, N. Y. In the present studies the plots are identified by the same numbers used in the other studies. On these plots, each of which covered from one-tenth to one-fourth acre, all essential details,

such as species, size, and position of each tree in the forest canopy, were recorded at each periodic examination.

To determine the effect of a reproduction cutting made in 1902, plots 321 and 323 were established in 1906 in a 45-year-old stand of second-growth hardwoods on the Maltby tract. All the chestnut

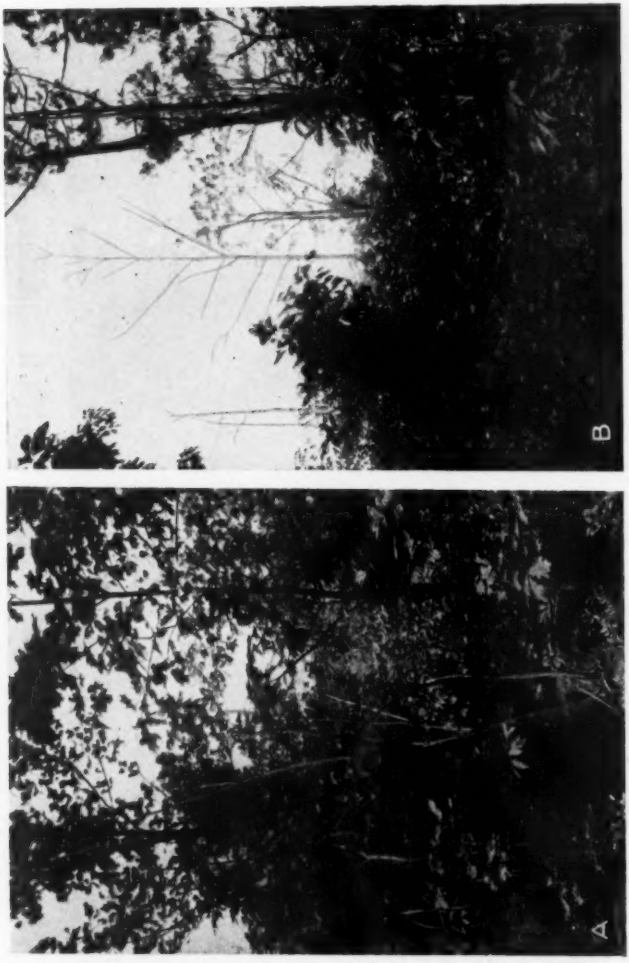

FIG. 2.—A, a young stand of hardwoods following clear cutting about 15 years before. The first three generations of chestnut sprouts have been killed by the blight and the overtopped fourth generation is heavily infected. The opening left by the chestnut has been completely filled by red oak, hickory, and yellow poplar, the crowns of which are interlaced. (Maltby tract, southern Connecticut). B, a mixed stand of hardwoods and pitch pine in Sussex County, N. J. The blight-killed chestnut is being replaced by a dense growth of young hardwoods containing some young pitch pine and white pine in mixture.

on these plots was cut and salvaged in 1913. Plot 474 was laid out in 1910 in a 45-year-old stand on the Whittemore estate. A heavy thinning ("C" grade) was made the same year. In 1915 an improvement cutting was made, taking out about 1 cord per acre. In this cutting, all of the dead and suppressed trees, most of the intermediate trees, and some of the codominant trees—those crowded

from the side but with their crowns still in the upper story of the stand—were cut. Oak was left wherever practicable because it was then evident that the chestnut would not survive the blight. Plot 475 was located in the same stand, but received no special treatment

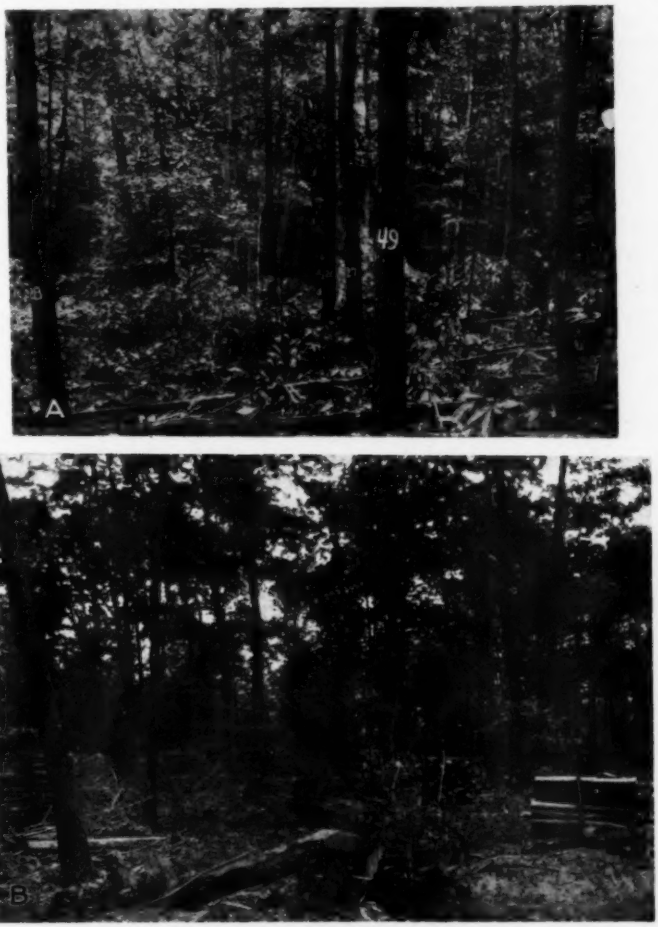

FIG. 3.—A, permanent sample plot in 1910, thinned to stimulate the growth of the stand. B, the same plot in 1924 after the blight-killed chestnut had been salvaged and the remaining stand improved by cutting out some of the less desirable individuals of other species. The material removed was used for railroad ties and charcoal

until the chestnut was salvaged in 1917–18 along with that on plot 474.

Plot 2 was laid out in 1910 in a 45-year-old stand of second-growth hardwoods on the Meshomasick State Forest, in which very little or no cutting had been done prior to that time. (Fig. 3.) Plot 4 was

situated in the same locality, but in a 25-year-old stand. The first important cutting on either of these plots was made in 1924. It consisted of a chestnut salvage cutting combined with a light improvement cutting applied to the remaining stand, which ran heavily to oak. The main objects of the improvement cutting were to leave the area in the best possible condition and to favor red oak, chestnut oak, white ash, and hickory, and at the same time to keep down the proportion of red maple and other less desirable species.

At the time these plots were established the respective percentages of chestnut in the stand on the basis of basal area were as follows:

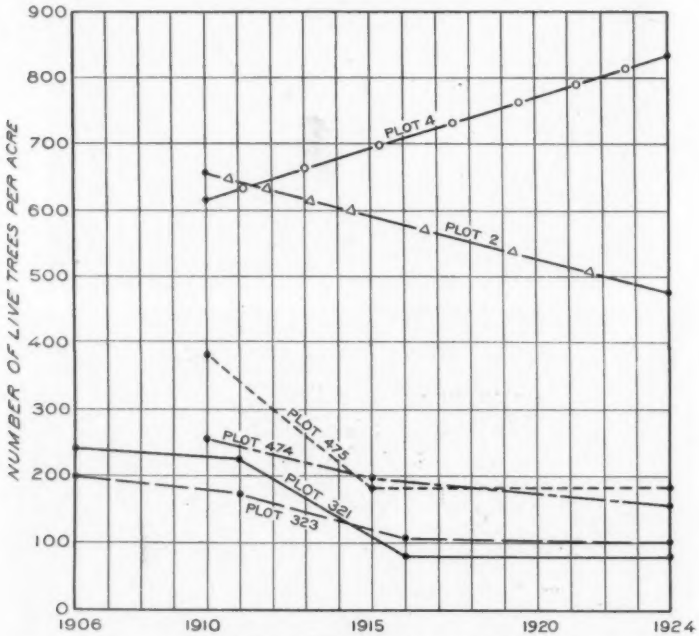

FIG. 4.—Progressive change in number of live trees per acre (1906–1924), due mainly to heavy mortality in chestnut between 1911 and 1916

Plot 321, 50; plot 323, 32.7; plot 474, 71.2; plot 475, 66.6; plot 2, 42.7; and plot 4, 44.2. At the last examination of these plots (in 1924) all essential details, such as species, size, and position of each tree in the forest canopy, were again recorded. After computing the detailed results of each successive examination it was possible to trace the history of the various changes taking place in the forest during the last 13 to 18 years. For each plot the progressive changes in total number per acre of live trees of all species 1 inch or more in diameter is shown in Figure 4. The change in basal area per acre of all live trees is shown in Figure 5.

Figure 5 shows the material decrease in basal area occurring between 1911 and 1915, when the chestnut dropped out of most of the stands. It must, however, be recalled that the improvement cuttings which

removed some material other than the chestnut also reduced the growing stock to some extent. Plots 321 and 323 have shown a slight increase in basal area since 1916 in spite of the fact that a slight decrease in number of trees occurred. It is not surprising to find stands in which the basal area is still decreasing, even though the number of trees may be increasing. This is well illustrated by plot 4, in which the death of a small number of large chestnuts more

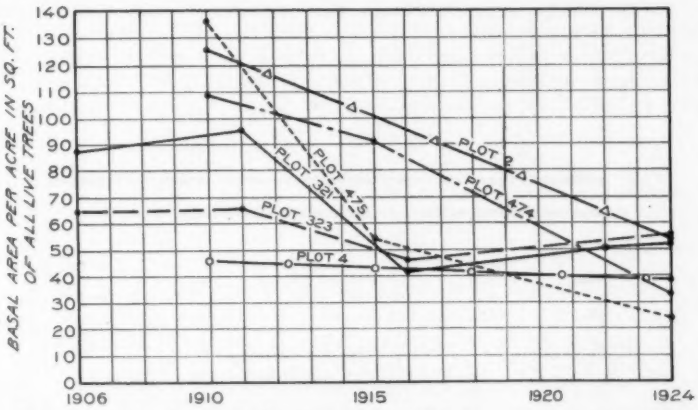

FIG. 5.—Progressive change in basal area per acre (1906–1924), due mainly to heavy mortality in chestnut between 1911 and 1916

than offset the gain in basal area of a large number of small trees which had just entered the measurable size class.

In order to generalize the results from these plots and bring out the significant changes caused by the chestnut blight, the data for all have been combined on an acre basis in Table 1, which summarizes

TABLE 1.—*Average number of live trees and average basal areas per acre for the dominant stands* [a]

Species	1910–11				1924			
	Trees per acre		Basal area		Trees per acre		Basal area	
	Number	*Per cent*	*Square feet*	*Per cent*	*Number*	*Per cent*	*Square feet*	*Per cent*
Chestnut	153.3	70.5	64.18	76.2	0	0	0	0
Red oak	22.0	10.1	9.13	10.8	38.0	28.6	16.45	42.2
Chestnut oak	22.0	10.1	5.25	6.2	52.7	39.7	14.48	37.2
White oak	9.4	4.4	1.97	2.3	9.4	7.1	2.07	5.3
Black oak	2.0	.9	.82	1.0	3.3	2.5	.94	2.4
Scarlet oak	8.0	3.7	2.75	3.3	5.3	4.0	3.31	8.5
Sweet birch	.7	.3	.13	.2	0	0	0	0
Red maple	0	0	0	0	8.7	6.6	.82	2.1
White ash	0	0	0	0	6.7	5.0	.32	.8
Hickory	0	0	0	0	6.0	4.5	.29	.8
Sugar maple	0	0	0	0	2.7	2.0	.26	.7
Total	217.4	100.0	84.23	100.0	132.8	100.0	38.94	100.0

[a] Includes dominant and codominant trees on permanent sample plots 321, 323, 474, 475, 2, and 4. Basal or cross-sectional area measured at 4.5 feet above ground.

the number of live trees and basal areas of the dominant stand as they were in 1910–11 and in 1924. The change in average basal area for the same six Connecticut plots is shown graphically in Figure 6. The outstanding feature of Table 1 is the increase in basal area of red oak and chestnut oak, together with an actual increase in the number of stems. The natural replacement of the original chestnut forests by stands running very largely to valuable oak is strikingly brought out by this table. While these plots were located in typical stands, some had been thinned one or more times before the 1910–11 records were taken. In 1910 the 217.4 trees per acre in the upper crown class ranged in size from 3 to 13 inches, breast high, averaging 8 inches; and now after the chestnut has died and the forest is beginning to rehabilitate itself, there are 132.8 trees per acre

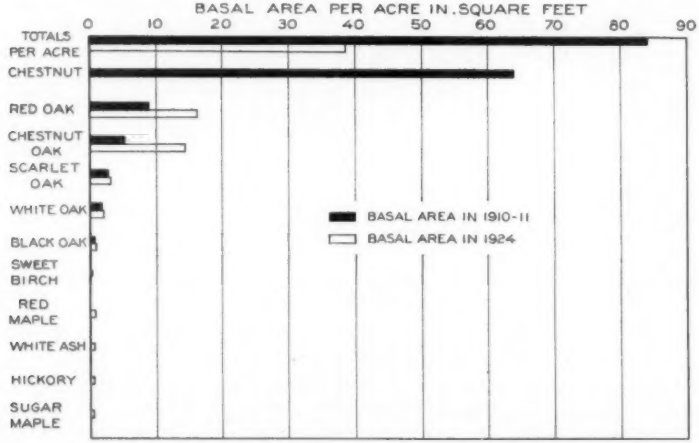

FIG. 6.—Change in basal area by species for the dominant part of the stand from 1910–11 to 1924 (graph based on averages from summary of six Connecticut plots)

in the upper crown cover, ranging from 2 to 15 inches in diameter and averaging 7.3 inches.

Permanent plots 319 and 320 are of particular interest. They are located on the Maltby tract in a stand which was clear cut about five years prior to their establishment in 1904. In 1909 chestnut comprised 24 per cent of the stand, while in 1924 the only living chestnut was sprout growth an inch or less in diameter and not more than 3 to 4 years old—overtopped, heavily infected, and dying. This area is now stocked at the rate of 785 trees to the acre in the upper crown cover, ranging from 1 to 6 inches in diameter.

The dominant stand on these plots is composed of 45 per cent class 1 species, 41 per cent class 2 species, and only 14 per cent class 3 species. Out of a total of 995 trees of class 3 species, 89 per cent are overtopped trees, which on account of their slow growth will probably not gain a position in the upper crown cover. In fact, some of these trees now in the overstory will in time be overtopped by the faster-growing species of classes 1 and 2.

A study of Figure 7, a sketch of the crown spread of the trees on permanent plot 320 in 1904, and Figure 8, a sketch of the same plot in 1924, clearly shows that the chestnut has completely disappeared from the dominant part of the stand, and that the crown cover of the dominant stand is already almost completely closed. From the standpoint of crown closure, this plot shows exceptionally good re-

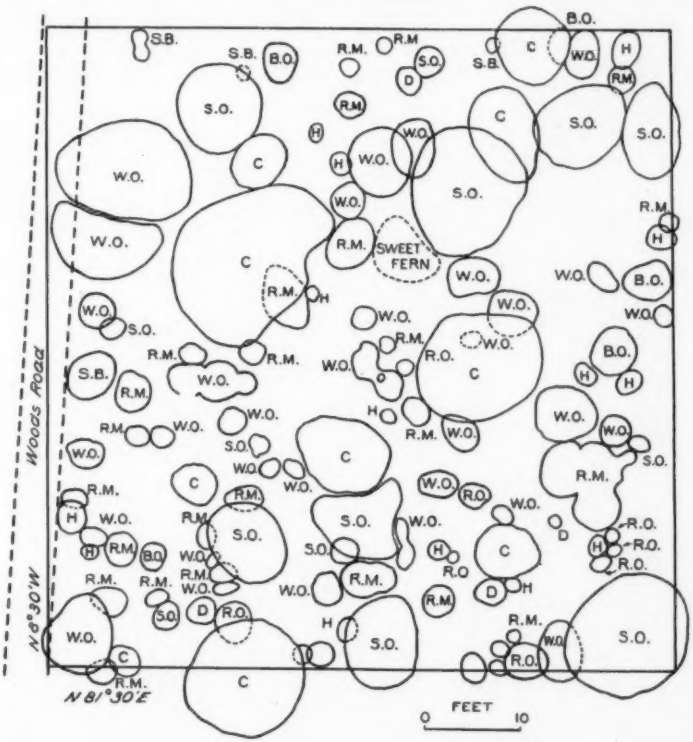

FIG. 7.—Crown spread of trees on permanent sample plot No. 320, as sketched in 1904 (Maltby tract)

covery. The amount of red maple in the upper crown cover of this stand is above the average for otherwise similar stands. Its preponderance is probably due to the fire which lightly burned over the area a few years after cutting. The fire favored the increase of red maple in the overstory, and dogwood in the understory. However, on sites of second quality, similar to this area, red maple is much less aggressive than it is on the better sites (*10*).

The decrease in the number of trees on most of the permanent plots is due not only to the loss of chestnut, but also to the removal

of some of the less vigorous and less desirable individuals of the oaks and other species when the chestnut was salvaged. This reduction in forest growing stock or capital is partly offset by the increased growth of the oaks. Furthermore, it must be remembered that the openings left in the forest as a result of the death of the chestnut contain a significant number of seedlings and sprouts, mostly of the valuable species as yet too small to appear in the plot records. (Fig.

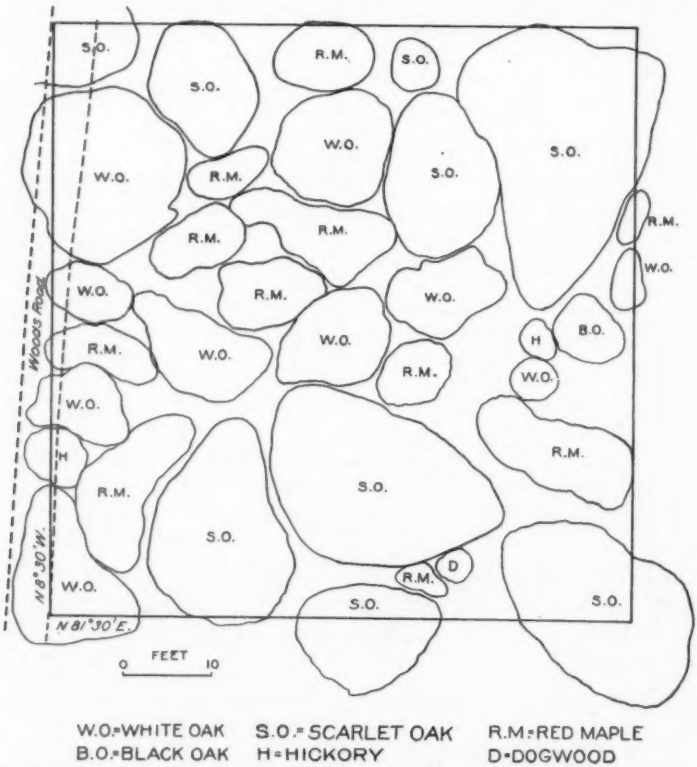

FIG. 8.—Crown spread of the stand on the same plot shown in Figure 7, as sketched in 1924. Because of the almost complete closure of the dominant stand, the overtopped trees were not mapped. The chestnut has completely disappeared from the overstory

9.) The results of a special study, aimed to determine specifically the nature and extent of restocking in the openings formerly occupied by chestnut, will be given on a subsequent page.

STUDIES ON TEMPORARY PLOTS

Records were made of the stands on 1 quarter-acre and 3 tenth-acre plots, and of the reproduction on 24 square-rod plots on the Maltby tract. The quarter-acre plot (No. 1) was laid out in a 60-year-old stand which contained about 35 per cent chestnut before it was killed by the blight. The dead chestnut poles are still standing

and have sprouted very feebly. The dominant stand contains 96 trees to the acre, of which 96 per cent are class 1 species, 4 per cent are class 2, and none are undesirable. Red oak and white oak between 5 and 14 inches in diameter form over 87 per cent of the

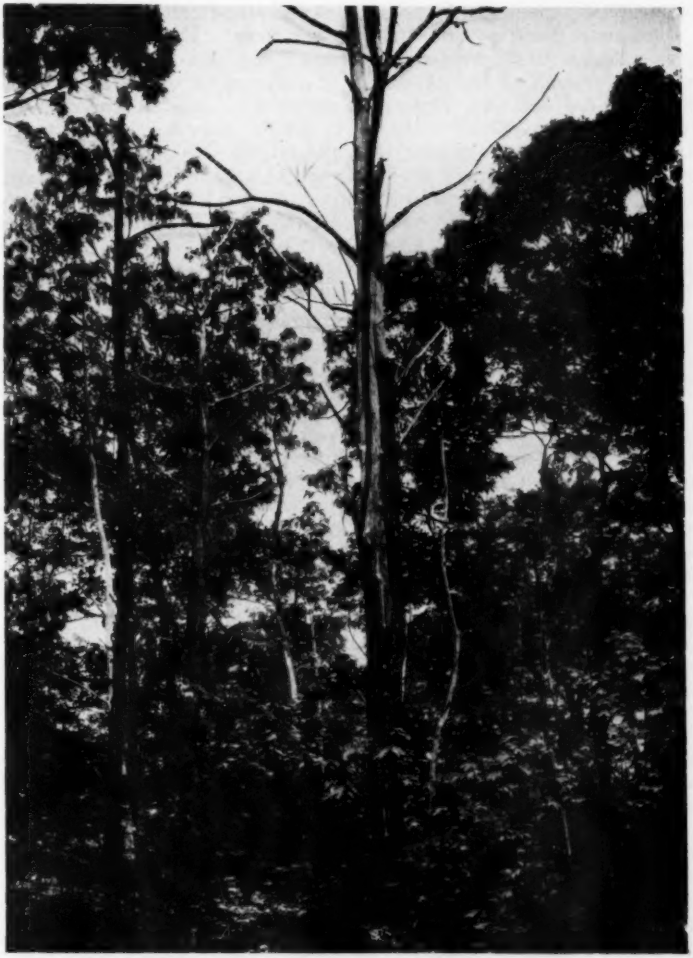

FIG. 9.—A blank in a stand of hardwoods left by the death of the chestnut. The surrounding oaks are filling it by an extension of their crowns. The crown cover will soon be completely closed. (Maltby tract, southern Connecticut)

dominant stand. The undesirable species are practically confined to the understory. The dominant stand is reinforced by a subordinate stand of 572 trees, of which 46 per cent are class 1 species and 15 per cent are class 2. It is therefore evident that although this

stand is somewhat depleted, conditions are favorable for a satisfactory restocking.

Plots 2 and 3 were tallied in a 15-year-old stand which promptly followed clear cutting. The area had probably not been burned over since the cutting and received no subsequent treatment. Chestnut originally made up 30 per cent of the stand. The area now bears a dominant stand of 835 trees to the acre. In this stand class 1 species lead with 65 per cent, and class 2 species comprise 19 per cent; only 16 per cent of the overstory is made up of undesirable species. Here also red oak and white oak are preponderant in the overstory, with smaller quantities of hickory, beech, gray birch, sweet birch, black oak, red maple, scarlet oak, largetooth aspen, white ash, sugar maple, and basswood, in the order of their abundance. The understory contains 88 per cent of the total number of undesirable species. On the other hand it also has 1,075 trees of class 1 species, or 43 per cent of the total number, and 455, or 18 per cent, of class 2 species—more than enough to insure restocking with the better species.

Plot 4 was tallied in a stand that had been thinned quite heavily in 1906. All the chestnut was removed in 1913; the oaks were left until 1921, when they, too, were cut. The resulting stand, on an acre basis, has a total of 2,590 saplings, at present receiving practically full light from above. Of these, 32 per cent are class 1 species, 54 per cent class 2 species, and only 14 per cent class 3 species. Since red maple constitutes 34 per cent and dogwood 9 per cent of the overstory, cleanings or thinnings may be advisable to increase the proportion of the more desirable species in this stand. A similar operation in a permanent sample plot is shown in Figure 3. Scarlet oak, red oak, hickory, white oak, sweet birch, sugar maple, and beech follow in the order of relative abundance. These vary from 330 scarlet oak saplings to 110 beech. Of the class 1 species 72 per cent are in the overstory. Of the undesirable species 53 per cent are already overtopped. In the overstory 60 per cent of the class 1 species, 17 per cent of the class 2, and 19 per cent of the class 3 species are of seedling and the remainder of sprout origin. The combination of desirable species and desirable (seedling) origin is particularly noteworthy.

All the plots considered thus far fail to bring out clearly the nature and extent of replacement within the gaps left by the chestnut and the proportion of shrub or inferior species which may be taking the place of the valuable chestnut. To obtain this information 24 square-rod sample plots were laid out and tallied in Connecticut in 1924, and records were made of the forest-tree reproduction and of the larger or more abundant species of shrubs above 6 inches in height. Each plot was laid out so that the center of the clump of dead chestnut formed the center of the plot. Plots were tallied in four representative stands, varying from recently clear-cut stands (plot 4 above) to a 60-year-old stand in which the merchantable chestnut had not yet been salvaged (plot 1 above). The records of these plots are summarized in Table 2. Attention should be directed to the fact, not brought out in the table, that the live chestnut sprouts seldom attain a diameter of more than an inch. The third and fourth generations of sprouts were being killed by the blight at the time the tallies were made. Two or three sprout generations may still be expected on the

clear-cut areas and on those on which the chestnut was salvaged before it died. Many of the clumps of chestnut which were not cut before the trees died have already ceased to sprout.

TABLE 2.—*Reproduction under blight-killed chestnut, southern New England* [a]

Plots			Chestnut reproduction per square rod			Other reproduction per square rod [b]					Percentage of area on which tallest saplings are found	
							Class 1 species		Class 2 species			
Nature	Number	Average diameter of chestnut clumps	Dead sprouts	Live sprouts	Live seedlings	All species	Sprouts and seedlings	Proportion of seedlings	Sprouts and seedlings	Proportion of seedlings	Class 1 species	Class 2 species
		Feet	*Number*	*Number*	*Number*	*Number*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Stand clear-cut in 1921 (plot 4)	8	11.4	17.1	11.0	0.6	44.6	32	55	24	11	60	27
Stand clear-cut in 1908 (plots 2 and 3)	6	8.0	17.0	7.9	.0	24.7	60	74	18	15	79	0
Permanent plot 474 [c]	5	------	------	28.0	1.6	93.8	75	59	11	45	------	------
60-year-old stand (plot 1)	5	19.3	16.0	10.6	.0	43.4	41	69	3	57	67	33
Weighted averages	------	12.4	16.8	13.7	.5	49.6	49.8	63.5	15.4	28.7	67.8	20.1

[a] Summaries of tallies of 24 square-rod reproduction plots, grouped by stands of essentially similar age or treatment; Maltby tract, near New Haven, and Whittemore estate, near Woodbury, Conn.

[b] Class 1 species are: White oak, red oak, black oak, chestnut oak, white ash, hickory, sweet birch, black cherry, basswood, sugar maple, and yellow poplar. Class 2 species are: Scarlet oak, red maple, beech, and aspen. Class 3 species are included only in the total number of all species.

[c] For description see preceding section of text.

The most striking point brought out by Table 2, aside from the fact that the blanks are now filled by a much larger number of seedlings and sprouts than can possibly survive, is that practically half of the new reproduction is of desirable species and that about 65 per cent of these are species which at present have an economic value. Furthermore, on over two-thirds of the area represented by the plots the tallest saplings are class 1 species, and on another 20 per cent they are class 2 species. Over 63 per cent of the class 1 reproduction is of seedling origin.

The goodly portion of red oak in the replacement reproduction on so many of the plots is especially noteworthy. The importance of red oak, because of its rapid growth in height and diameter and the excellent character of its timber, has long been recognized by foresters (*3, 6*). Spaeth (*15*) has classed both red oak and white ash as important species in replacing chestnut. There is little doubt that these species will increase rapidly in importance now that their formidable rival—the chestnut—has been destroyed. The second-growth hardwood type will be easy to maintain and will form the basis of future forest management on the better soils.

The most significant facts brought out by the 1924 Connecticut studies, as well as by those made in 1920, are that there is generally sufficient advance growth of valuable species to restock the stands, and that the blight-killed chestnut has been very largely replaced by

oak. (Fig. 1.) On areas originally occupied by chestnut, oak reproduction was found very generally, together with reproduction of such other valuable species as hickory, white ash, sugar maple, and sweet birch. (Fig. 2, A.)

These studies, made under representative conditions, indicate that the blight-killed chestnut stands of southern New England are restocking naturally to silviculturally desirable and economically valuable species. These species are much in excess of the undesirable shrub-weed species. If, as is occasionally true, the more desirable species are outnumbered by the undesirable ones, they nevertheless, having the more rapid growth rate, will soon overtop the inferior species and partially or completely suppress them. It is also encouraging to note that in those cases where the total number of desirable species may not equal that of the undesirable ones, the desirable species in the overstory are already in excess of the others. Moreover, the tallest saplings in the gaps left by the death of the chestnut are mostly of desirable species, such as the oaks, hickory, and ash, rather than of shrub or weed species, such as dogwood, sassafras, blue beech, and gray birch.

REPLACEMENT IN NEW JERSEY

Since no permanent sample plots were available for study in New Jersey, it was necessary to resort wholly to temporary plots. Three quarter-acre plots were laid out in uneven-aged stands on the Stokes State Forest, in Sussex County, northwestern New Jersey. Chestnut, at the time of its death, comprised 30 to 60 per cent of the stand. In this locality the chestnut had been dead a shorter time (mostly less than five years) than in the regions to the south and east. The first generation of sprouts had died, and the second generation was appearing.

Plot 1 is located on a moist flat in what was originally an oak-chestnut-pine forest. The overstory of the stand contains 1,316 trees to the acre, of which 12 per cent are class 1 species, 40 per cent class 2 species, and 48 per cent class 3 species. The class 1 species, in the order of their abundance, are white oak, chestnut oak, and white pine, while the class 2 species, in the order of their importance, are red maple, scarlet oak, black gum, and largetooth aspen. In addition to these, the overtopped stand contains about 600 trees to the acre, some of which are capable of increased growth when released, or of filling the gaps left by the chestnut.

Plots 2 and 3 are located on a low ridge in an uneven-aged stand which originally was an oak-chestnut forest. These plots have an average stand of 1,879 trees to the acre in the overstory, of which 34 per cent are class 1 species, 18 per cent are class 2 species, and 48 per cent are class 3 species. Of the desirable species over 60 per cent are in the upper crown class. These stands are obviously in need of an improvement cutting aimed especially at increasing the proportion of desirable species, which was reduced about 20 years ago by a cutting that culled out most of the best pitch pine. Reproduction of desirable species is rather deficient because of the heavy cover of mountain laurel (*Kalmia latifolia*) and scrub oak. These undesirable species will in time be shaded out by the tree species, the scrub oak being the first to give way. With protection from fire the scrub oak is very short lived, for on account of its intolerance of

shade it can not compete with any tree growth which overtops it (*1*). If the stand is allowed to develop without artificial treatment, it will eventually become an almost pure stand of the better species of oak, with some maple and a small quantity of pitch pine. It will, however, be more irregular, less well stocked with the better species, and slower in growth rate than with treatment. Although the pine is here somewhat more aggressive as a replacement species, particularly in filling larger openings left by the death of the chestnut, many of the pine seedlings are lacking in vigor and will probably be overtopped by the hardwoods.

Fifteen reproduction plots similar to the 24 small plots in the New England study were also laid out, the center of the plot coinciding with the center of the chestnut clump. The results obtained on these plots are summarized in Table 3. It is evident that there is an ample supply of forest-tree reproduction to fill the gaps, although the percentage of class 1 species is much lower than was found in southern New England. With less than half of the reproduction of species other than chestnut falling in classes 1 and 2, the desirability of modifying the composition of the stand is emphasized. It is encouraging, however, to note that the tallest saplings on slightly over 80 per cent of the area represented by the plots are either class 1 or class 2 species, although the percentage of class 1 is still small.

The stands studied in New Jersey were characterized by the presence of white pine and pitch pine, and by a higher percentage of the undesirable species in the overstory and a denser understory and undergrowth than those studied in Connecticut. (Fig. 2, B.) Although the chestnut is being replaced naturally for the most part by tree species, yet the presence of a relatively high proportion of undesirable species introduces important problems in connection with subsequent thinnings and improvement cuttings.

TABLE 3.—*Reproduction under blight-killed chestnut, New Jersey* [a]

Plots		Average diameter chestnut clumps	Chestnut reproduction per square rod			Other reproduction per square rod [b]					Percentage of area on which tallest saplings are found	
							Class 1 species		Class 2 species			
Nature	Number		Dead sprouts	Live sprouts	Live seedlings	All species	Sprouts and seedlings	Proportion of seedlings	Sprouts and seedlings	Proportion of seedlings	Class 1 species	Class 2 species
Uneven aged; oak-chestnut forest; flat ridge (plots 2 and 3)	10	*Feet* 20.4	*Number* 18.8	*Number* 20.8	*Number* 2.1	*Number* 55.2	*Per cent* 23	*Per cent* 96	*Per cent* 22	*Per cent* 84	*Per cent* 20	*Per cent* 56
Uneven aged; oak-chestnut-pine forest, flat (plot 1)	5	18.5	13.6	22.4	.6	45.8	13	61	34	75	27	64
Weighted averages		19.8	17.1	21.3	1.6	52.1	19.7	84.3	26	81	22.3	58.7

[a] Summaries of 15 square-rod plots tallied on the Stokes State Forest in northwestern New Jersey.

[b] Species in the order of their abundance are: Class 1, red oak, black oak, chestnut oak, white oak, hickory, sweet birch, white pine, and pitch pine; class 2, scarlet oak, red maple, black gum, and aspen; class 3 species are included only in the total number of all species.

An earlier sample-plot field study, made by Richards (*14*) in Somerset County, N. J., is of interest with respect to both his results and conclusions, the conclusions being at variance with those of the present studies. Chestnut oak was found to be the predominant species in the reproduction, amounting to 44 per cent on the ridge tops and 22 per cent on the slopes. On the ridge tops 11.8 per cent was hickory, 6.8 per cent red oak, and 3 per cent white ash. These four were the most valuable species, and comprised 65.6 per cent of the reproduction on the ridge tops. The remainder was made up of red maple, cherry, butternut (*Juglans cinerea*), birch, and sassafras. Reproduction on the slopes, in addition to chestnut oak, was made up of 6 per cent red oak and 10 per cent pignut hickory (*Hicoria glabra*), besides some cherry, maple, and butternut.

Because the desirable species, other than chestnut oak, were in the minority, Richards concluded that there was little reproduction in the way of desirable native species with which to form the new forest, and consequently recommended replacement of the chestnut by planting Norway pine (*Pinus resinosa*). However, Richards's study was mostly of areas cut over less than a year previously (a few of his plots had been cut over seven years), so that in all probability most of the reproduction he found was advance growth and much of the seedling growth prominent in the replacement had not yet become established. Had his study been made a few years later, on areas cut over for a longer time, he would probably have found more reproduction and a higher proportion of desirable species, and accordingly less occasion for the planting of pine.

REPLACEMENT IN PENNSYLVANIA

The studies of natural replacement were not so intensive in Pennsylvania as in Connecticut and New Jersey, since this problem was already being studied by J. S. Illick and assistants, for the Pennsylvania Department of Forests and Waters.

Two sixteenth-acre plots laid out near Lehigh Gap, Pa., in 1920, are of special interest. One plot was on an area which, at the time it was clear-cut three years before, contained 62 per cent chestnut. The new stand contained 9,424 live trees to the acre, of which 22 per cent were chestnut sprouts. Of the remaining live trees, 86 per cent were class 1 species, chestnut oak, white oak, and sweet birch; 14 per cent were class 2 species, scarlet oak, red maple, black gum, and cottonwood; and none were undesirable species. The other plot was located in a stand cut heavily for chestnut 25 years earlier. This stand originally included 93 per cent chestnut, but in 1920 the proportion of chestnut sprouts in the live stand had been reduced to 32 per cent, and by 1924 the chestnut had been entirely eliminated. The stand contained a total of 10,234 trees to the acre, exclusive of chestnut, of which 40 per cent were class 1 species, white oak, black oak, sweet birch, red oak, black cherry, and hickory; 55 per cent were class 2 species, red maple, scarlet oak, black gum, and cottonwood; and only 5 per cent were undesirable. These stands have obviously restocked mostly to desirable species, and almost as rapidly as the chestnut disappeared from the stand.

Stand records, obtained in company with T. E. Shaw, of the State Forest School in the Mont Alto Forest in Franklin County, show that on slopes where the chestnut had occupied 40 per cent or more of the original stand seedlings and sprouts of other species varied from 1,000 to 1,500 per acre. Of these, chestnut oak comprised 60 per cent, black oak 35 per cent, and white oak 4 per cent. Scattered red and scarlet oaks, white and pitch pines, and black locusts made up the remainder. Here the oaks, which in the original stand were second in number to the chestnut, have insured adequately stocked stands of second growth. Chestnut oak, pitch pine, and black locust (*Robinia pseudoacacia*) are also among the desirable species which are largely replacing the chestnut in Pennsylvania. Black oak, pignut hickory, sweet birch, mountain pine (*Pinus pungens*), and Virginia pine (*P. virginiana*) are others which are helping to fill the gaps, and are not to be despised (*11*).

The Pennsylvania studies also showed that the blighted areas are being restocked by natural reproduction, mostly of desirable species. The replacement is more complete and more satisfactory in moist situations than upon the drier slopes, for blanks on the moist sites are relatively less extensive and the associated species, such as yellow poplar, white ash, red oak, white pine, red maple, and white oak, more desirable. Upon the drier slopes the blanks are more extensive, and the associated species are less desirable.

SUMMARY

This paper presents the results of studies to determine the character and amount of natural replacement of blight-killed chestnut. They were begun in the earlier infected chestnut stands of the Northeast, where natural replacement is most advanced and could most easily be studied.

In southern Connecticut permanent sample plots established between 1904 and 1910, as well as temporary plots, were studied. In New Jersey it was necessary to resort wholly to temporary plots. Limited studies were also made in Pennsylvania.

The natural replacement of the forests, originally containing large quantities of chestnut, by stands running very largely to oak—red oak, white oak, chestnut oak, black oak, or scarlet oak—is strikingly brought out by these studies. An outstanding feature is the increase of red oak and chestnut oak both in number of trees and basal area. Such other desirable species as hickory, white ash, sugar maple, and sweet birch may be associated with the oak.

While the stands are for the most part still depleted in basal area they are recovering satisfactorily. From the standpoint of crown closure the areas studied also showed exceptionally good recovery. The stand remaining after the death of the chestnut, composed chiefly of oaks, has responded to increased light, and the crowns of these trees are very effectively closing the smaller openings left by the chestnut.

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ISOLATION AND IDENTIFICATION OF SOME ORGANIC NITROGENOUS COMPOUNDS OCCURRING IN ETIOLATED CORN SEEDLINGS[1]

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INTRODUCTION

There are in the literature many records concerning the occurrence of asparagine in plants. These records refer chiefly to legumes, although other plants, too, were found to contain that amino acid. Thus, as early as 1867, Beyer (*1*)[2] found asparagine in seedlings of the yellow lupine while von Gorup-Besanez (*3*, *4*) reported its occurrence in vetch seedlings. The finding of Beyer was corroborated by Schulze (*14*), by Schulze, and Umlauft (*21*), as well as by Schulze and Barbieri (*17*), while the report of von Gorup-Besanez was confirmed by Schulze, Steiger, and Bosshard (*22*). From the extensive work of Schulze and his collaborators we know also that asparagine is found in potato tubers (*16*), young lucern plants (*15*), oat plants (*22*), etiolated soy-bean seedlings (*13*), asparagus, celery, dahlia bulbs (*15*), and other plants (*18*). Frankfurt has also reported the occurrence of asparagine in the wheat embryo (*2*). Of the cereals only the wheat embryo and young oat plants have been reported to contain asparagine, as far as the writer is aware. After it had been shown in a previous paper (*9*) that a great part of the proteins of the maize kernel is converted into amino acids, polypeptides, and other degradation products by the process of germination, it was of interest to find out the exact nature of the individual cleavage products, since the latter differ from each other in many respects from the standpoint of nutrition and physiology. It was to acquire this additional information that the present investigation was undertaken. In it the occurrence of asparagine and vernin in etiolated corn seedlings is reported.

METHODS AND RESULTS

PRELIMINARY ANALYSES OF SEED

Prior to starting germination experiments it was thought advisable to secure some data regarding the composition of the seed. Accordingly, several determinations were made, with the results recorded in Table 1.

[1] Received for publication Jan. 31, 1927; issued May, 1927.
[2] Reference is made by number (italic) to "Literature cited," p. 655.

TABLE 1.—*Proportion of moisture and ash, and of total nitrogen, protein nitrogen, and nonprotein nitrogen in the ungerminated corn kernel*

Variety	Moisture in—		Ash in—	Total nitrogen in—	Protein nitrogen in—		Nonprotein nitrogen in—	
	Air-dry kernels	Air-dry flour	Oven-dried kernels	Oven-dried flour	Oven-dried flour	Total nitrogen	Oven-dried flour	Total nitrogen
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Iodent (1924 crop)	11.48	9.15	1.57	1.67	1.56	92.81	0.18	10.57
Do	11.27	9.35	1.39	1.69	1.53	90.97	.17	9.92
Do			1.48	1.67	1.57	93.20	.14	8.53
Average	11.38	9.25	1.48	1.68	1.55	92.33	.16	9.67

In connection with Table 1 it may be mentioned that the flour represents the whole maize kernel, which was ground finely enough to pass a 40-mesh sieve. A moisture determination of the kernel was made in order to have a basis for the calculation of the results obtained on the oven-dried kernel. The protein nitrogen was determined by the Stutzer method, referred to in previous papers (*8*, *11*).

METHODS OF GERMINATION

The seed (Iodent variety) was spread on perforated metal trays, ordinarily from 350 to 400 kernels per tray, between moist absorbent paper towels, and allowed to remain in a dark germinator at a temperature of from 25° to 27° C. The seedlings were usually watered once a day with distilled water. At the expiration of five to seven days the sprouts were severed from the kernels and dried in an electrically-heated oven at 55°–60° for about two days, and then ground and passed through a 40-mesh sieve.

EXTRACTION

Ordinarily, 100 gm. portions of the flour were treated with boiling ammonia-free water, shaken well and heated on the steam bath for at least 15 minutes. The whole was now centrifuged or filtered on a Büchner funnel with suction. The solid residues were extracted once more in like manner. To the combined extracts subacetate of lead was added as long as a precipitate came down. A slight excess of the precipitant was used. The precipitate was allowed to stand 24 hours, at the expiration of which it was filtered off with suction and washed with water.

ISOLATION

Filtrate and washings were now treated with a solution of mercuric nitrate. After this treatment the whole was made neutral to litmus with sodium hydroxide solution and allowed to stand for 24 hours. When the precipitation was complete the precipitate was collected on a filter and washed with cold water. The washed precipitate was then suspended in water and decomposed with hydrogen sulphide. The mercuric sulphide precipitate was filtered off and the clear filtrate neutralized carefully with a few drops of strong ammonia and evaporated on the water bath at low temperature to a thin sirup a drop or two of ammonia being added from time to time to keep the reaction

neutral. When sufficiently concentrated the substance was allowed to solidify slowly in a vacuum over sulphuric acid. It was noticed that with increasing concentration the sirup gradually assumed a dark, almost black color, being so sticky that it could not be removed from the crystallizing dish. The sirup was readily soluble in water and practically insoluble in alcohol. When alcohol was added to the aqueous solution a dark brown sediment gradually formed at the bottom of the vessel, the supernatant liquid becoming lighter in color. When spread on porous, unglazed porcelain the sirup gradually became free from the sticky substance. The whole of the sirup was purified by taking advantage of the two observations made.

After the solid substance had been removed from the unglazed porcelain it was dissolved in hot water, filtered, and allowed to concentrate and crystallize in vacuo over sulphuric acid. The almost dry, impure, crystallized mass obtained weighed 16 gm. It was procured from 1,200 gm. of air-dry corn sprouts whose ungerminated kernels had the composition shown in Table 1. After several recrystallizations from hot water the fair-sized crystals, which could be picked out without difficulty, became clear and practically colorless. They had the form of rhombic prisms and will be referred to in this paper as "Crystals A." On removal of these crystals the remaining substance was dissolved in hot water, filtered, and again allowed to crystallize in a vacuum over sulphuric acid. This mother liquor was expected to give another crop of "Crystals A." However, the crystals now obtained differed from "Crystals A" in their solubility and other properties, having the form of long, fine needles. They will be referred to as "Crystals B." The filtrate from these crystals gave another crop of "Crystals A," which, like the first crop, was purified by recrystallization from hot water.

IDENTIFICATION OF "CRYSTALS A"

A macroscopic and microscopic examination of the crystals showed them to have the form of rhombic prisms. They were hard, lustrous, translucent, and of considerable size when permitted to crystallize slowly. Some of them were about 7 mm. long. The crystals were easily soluble in hot water, difficultly soluble in cold water, and insoluble in alcohol and ether.

Chemical Reactions

A few crystals were dissolved in water and the following tests made:

(1) A portion of the solution was heated with dilute sodium hydroxide. When wet red litmus paper was exposed to the fumes it turned dark blue, showing the evolution of ammonia.

(2) Another portion of the solution, heated with dilute hydrochloric or sulphuric acid, neutralized with sodium hydroxide, and tested with Nessler's reagent, gave at once a reddish-yellow precipitate, characteristic of ammonia.

(3) Neutralized formaldehyde added to the neutral aqueous solution of the substance produced an acid reaction.

(4) An acid solution of the substance was dextrorotatory, while an alkaline solution was levorotatory.

(5) When treated with Millon's reagent the solution remained perfectly clear and colorless, showing that the substance was free from tyrosine.

The evolution of ammonia, when a solution of the isolated substance was heated with sodium hydroxide (reaction No. 1), may have been caused by the presence in it of an acid amide or an amino acid group. To decide this question definitely, reaction No. 2 was made. Since amino acids are resistant to the action of dilute acids, reaction No. 2 plainly shows that the isolated substance has an acid amide group. On the other hand, reaction No. 3 shows conclusively that the substance has also an amino acid group. The further fact that the substance is optically active (reaction No. 4) indicates that the amino group is very likely in the α-position, since all α-amino acids, with the exception of glycin, have an asymmetric carbon atom to which the optical activity is due. Inasmuch as mercuric nitrate is known to precipitate chiefly asparagine, glutamine, allantoin, nucleic bases, and tyrosine, and inasmuch as only asparagine and glutamine have both the acid amide and the amino acid group, the idea suggested itself that "Crystals A" might be asparagine or glutamine. This question was answered by the several quantative determinations given below.

Water of Crystallization

Two portions, 0.1585 and 0.2745 gm., respectively, dried at 103° C. to constant weight, lost 0.019 and 0.033 gm., corresponding to 11.99 and 12.02 per cent of moisture. Asparagine, $C_4H_8N_2O_3 + H_2O$, requires 11.99 per cent of water.

Specific Rotation

The specific rotation was determined with a half-shade polarimeter having a sugar scale, and the readings recalculated to degrees of circular polarization by the use of the factor 0.346. White light filtered through a dichromate cell was used. The following specific rotation tests were made:

(1) A quantity of "Crystals A" weighing 0.7505 gm. was dissolved in 25 c. c. of N/5 NaOH and made up with water to 50 c. c. Thus, a solution was obtained each cubic centimeter of which contained 0.01501 gm. of the crystals. This solution, observed in a 2 dcm. tube, gave α = observed rotation $-0.8°$ C.

(2) To 10 c. c. of the solution (= 0.1321 gm. of oven-dried crystals) 5 c. c. of N/5 HCl were added to neutralize the solution, after which 4.6 c. c. more of concentrated hydrochloric acid were added, a 10 per cent hydrochloric acid solution resulting. This solution, observed in a 2 dcm. tube, gave $\alpha = +1.5°$. Hence, $[\alpha]_D = +38.50°$.

(3) As a third test 0.2535 gm. of crystals (= 0.2231 gm. of oven-dried substance) was dissolved in a 10 per cent hydrochloric acid solution and made up with the acid to 15 c. c. The observation, in a 2 dcm. tube, gave $\alpha = +3.1°$. Hence $[\alpha]_D = +36.07°$. According to Schmidt (*12, p. 528*), $[\alpha]_D$ of asparagine in a 10 per cent hydrochloric acid solution is equal to $+37.45°$.

Nitrogen Determination

Two portions of 0.1 gm. each of the isolated crystals were oxidized according to Kjeldahl's method. They gave 18.490 and 18.570 gm. corresponding to 18.49 and 18.57 per cent, respectively, of nitrogen. Asparagine, $C_4H_8N_2O_3 + H_2O$, requires 18.67 per cent of nitrogen.

Formol Titration

For the principles underlying the formol titration method the reader is referred to the papers of Sörensen and his collaborators (*5, 6, 23, 24*), as well as those of the writer (*7, 10*). A quantity of "Crystals A," weighing 0.7505 gm., corresponding to 5 millimoles of asparagine, was dissolved in 25 c. c. of N/5 NaOH and made up with water to 50 c. c. Thus, a N/10 solution was obtained each cubic centimeter of which had received 0.5 c. c. of N/5 NaOH in advance of titration. Two 10 c. c. portions of this solution formol-titrated, with phenolphthalein as indicator, each required for their neutralization 0.03 c. c. of N/5 HCl, corresponding to 99.4 per cent of asparagine. Under similar conditions Sörensen (*23, p. 79*) found 99 per cent of asparagine. From 1,200 gm. of air-dry corn sprouts (5 to 7 days old) there were obtained about 12 gms. of pure asparagine, or 1 per cent of the total air-dry weight of the sprouts.

To sum up, the form of crystals and the chemical reactions, as well as the specific rotation, indicate the substance to be asparagine. Furthermore, the specific rotation and the formol titration, as well as the nitrogen and moisture estimation of "Crystals A," establish beyond any doubt that the isolated crystals are asparagine.

The considerable quantity of asparagine found in etiolated corn seedlings is noteworthy. After the proteins of the corn kernel have been decomposed to amino acids by the process of germination (*9*), the latter are gradually converted into asparagine, in accordance with Schulze's theory. As an amino acid, asparagine has direct nutritive value, but its physiological importance as a means of translocating nitrogen is of greater interest.

PARTIAL IDENTIFICATION OF "CRYSTALS B"

While the full identification of "Crystals B" will require considerable time and labor, a few preliminary data may not be out of place here. On concentration in a vacuum the filtrate from the first crop of asparagine gave crystals which, on being filtered off with suction, consisted of a light voluminous, brownish-yellow mass. The mother liquor from these crystals gave, on concentration, a second crop of asparagine whose mother liquor yielded a small portion of "Crystals B." Altogether about 1.2 gm. of these crystals was obtained.

Properties of the Crystals

The crystals (perhaps not perfectly chemically pure) are readily soluble in cold alcohol and ether, but insoluble in cold water, and difficultly soluble in hot water. The substance usually crystallizes from these solvents in long, fine, yellowish, microscopic needles, often arranged in raylike bunches. When allowed to crystallize slowly from hot water, it appears in fine, very long needles, some of which are more than 1.5 cm. in length. For purification the crystals were dissolved in absolute alcohol, filtered,[3] and concentrated on the water bath at low temperature. Long, light, silky, orange-yellow needles were formed. They have a slightly bitter taste and melt at about 142° C. to a brown-red liquid which solidifies at about 128°,

[3] The small residue on the filter contained asparagine.

and melt again at 142°. The solution of the crystals gives a strong positive reaction with Millon's reagent, indicating the presence of a free phenol group. The solution gives a negative reaction with Mörner's reagent, showing that the crystals were free from tyrosine. This substance was not further identified at this time.

IDENTIFICATION OF VERNIN

When the alcoholic solution of the second crop of "Crystals B" (see p. 653) was filtered off through a paper filter, there remained on the paper about 0.74 gm. of a substance which differed from both asparagine and "Crystals B." It had the form of an amorphous brownish-gray powder which was insoluble in alcohol and ether but readily soluble in water, especially in hot water. It was also soluble in dilute hydrochloric and nitric acids, as well as in dilute ammonia.

Crystallizability of the Substance

The brownish-gray powder was dissolved in hot water, filtered, concentrated on the water bath, and placed in a vacuum over sulphuric acid. It separated practically in the amorphous state. When the amorphous substance was treated again in the same manner, it was noticed that it gradually assumed the crystalline form. The impure substance showed under the microscope the presence of a few asparagine crystals. When it had been treated repeatedly with hot water, as outlined above, the substance was finally obtained as a crystalline mass. In the attempts to obtain the substance in the crystallized state it was observed that when the almost saturated solution of the substance was placed in a desiccator the whole of it solidified to a crystallized mass within a few minutes. To the naked eye the crystals appeared mostly as long, fine needles, while under the microscope long, water-clear, sometimes very narrow and thin prisms, imbedded in a brownish liquor, could be observed. The crystallized mass was mixed well with 95 per cent alcohol, filtered off with suction, and washed with alcohol.

Chemical Reactions

The aqueous solution of the substance was neutral to litmus paper. A solution of the substance in dilute hydrochloric acid, treated with phosphotungstic acid, gave immediately a yellowish-white precipitate. With silver nitrate the aqueous solution of the substance gave a characteristic white precipitate, soluble in ammonia. When some of the substance was dissolved in dilute nitric acid and evaporated on the water bath there remained a yellowish residue which, on being wetted with ammonia, assumed an intensified yellow color with a reddish tint. Judging from its description as given by Schulze and his collaborators (*19*, *20*, *22*), its solubility and crystallizability, the form of its crystals, and its reactions, the amorphous body is vernin. The workers mentioned reported this substance in young plants of vetch (*Vicia sativa*), clover (*Trifolium pratense*), pumpkin (*Cucurbita pepo*), ergot-infected rye (*Secale cornutum*) (*19*), and in the pollen grains of *Corylus avellana* and *Pinus silvestris* (*20*, *22*).

To further identify the amorphous body the crystalline material was thoroughly mixed with 95 per cent alcohol, filtered, and washed with alcohol. It was then dried in the air for 36 hours.

Quantities weighing 0.3800 and 0.1601 gm. of air-dry substance lost 0.0441 and 0.0184 gm. at 105° C. corresponding to 11.60 and 11.49 per cent, respectively, of water. The formula for vernin, $C_{16}H_{20}N_8O_8+3H_2O$, requires 10.68 per cent of water.

Quantities of oven-dried substance weighing 0.1410 and 0.1521 gm. oxidized according to the Kjeldahl method gave 0.0336 and 0.0361 gm., corresponding to 23.83 and 23.73 per cent, respectively, of nitrogen. The formula, $C_{16}H_{20}N_8O_8$, requires 24.78 per cent of nitrogen.

The analyses given suggest that the isolated vernin was not entirely pure. Unfortunately, a part of the substance, including a preparation of the silver compound, was lost by accident. However, as the preparation of more substance will require much time, it is deemed best to publish, in the meantime, the results obtained in this study by which the identity of vernin seems reasonably well established. Inasmuch as Schulze (*15*) has shown that vernin yields, on hydrolysis, a pentose and guanin, a hydrolytic product of nucleic acid found in the cell nucleus, it seems reasonable to assume that vernin may play a rôle in the synthesis of nucleoproteins.

SUMMARY

Asparagine has been shown to occur in etiolated corn seedlings. It was isolated and identified by its chemical reactions, by its specific rotatory power and its formol titration, as well as by its water and nitrogen estimation.

The occurrence of vernin in etiolated corn seedlings seems to be indicated.

A few preliminary data are given on what appears to be an orange-yellow pigment met with in etiolated corn seedlings.

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THE EFFECT OF APPLICATIONS OF CYANAMID ON THE NITRATE CONTENT OF FIELD SOILS[1]

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INTRODUCTION

Availability studies[2] with various nitrogenous fertilizers for cotton, conducted by the Fixed Nitrogen Research Laboratory, have indicated that in cases where cyanamid failed to give as good results as other carriers of nitrogen, one of the chief causes was the failure of the material to nitrify readily. Laboratory and greenhouse experiments[3, 4, 5] have contributed additional data along the same lines and have shown further that some of the products formed from cyanamid in the soil, particularly dicyanodiamide, are very toxic to the nitrifying bacteria. Experiments of numerous other investigators, discussed in the publications mentioned, have shown similar indications. In order to secure data on the rate of nitrate formation that might be more closely coordinated with the behavior of plants growing in the field, a series of nitrification studies was started in which soils from three of the experimental fields at Muscle Shoals, described in one of the publications cited [2] were used. These experiments were conducted at the same time that the cotton experiments were in progress and give a good idea of the nitrate content of the soils while the crop was growing.

PROCEDURE

The method of experimentation adopted was the usual procedure for nitrification experiments. The soils from the experimental fields were air-dried and 100 gm. samples weighed out into 200 c. c. beakers. To these soil samples were added the various fertilizing materials according to the scheme shown in the accompanying tables. This arrangement required 18 beakers, for each of the 6 soil samples used. Six samples were left untreated to serve as checks, 6 received 100 mgm. each of ammonium sulphate, and 6 received an equivalent amount of nitrogen as oiled and hydrated cyanamid. After the fertilizers had been thoroughly mixed with the soil, water was added to bring the moisture content up to the optimum, and the moisture content was maintained at this point throughout the experiment. The usual additions of calcium carbonate were omitted in order that the laboratory conditions for nitrate formation might more nearly approximate field conditions. Enough soil samples were used

[1] Received for publication Feb. 1, 1927; issued May, 1927.

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to allow for an analysis of duplicate samples at 3 different incubation periods. The experiment was started September 24, 1921, and analyses were made after incubation periods of 25, 42, and 61 days, respectively, at room temperature. The phenoldisulphonic acid method for determining nitrates was used in preference to the reduction method, because of the interference of the various decomposition products of cyanamid in the accuracy of the latter method.

RESULTS WITH SOILS FROM FIELD NO. 1

The soil in field No. 1 is a very poor hilly loam, slightly acid, especially low in phosphorus and nitrogen but capable of producing large crops of cotton when properly fertilized. Where cyanamid together with phosphates was used on this soil as a fertilizer for cotton, a marked retarding of early growth was noted, but to a lesser extent than on other fields. Usually the yields were nearly as good as with ammonium sulphate and sodium nitrate. The results of nitrification studies with this soil are given in Table 1.

TABLE 1.—*Nitrate content of soils from field No. 1 after fertilization with cyanamid and ammonium sulphate*

Treatment in field	Treatment in laboratory	Number of milligrams of nitrogen used per 100 gm. of soil	Quantity of nitrates present after 25 days (milligrams of N per 100 gm. of soil)	Quantity of nitrates present after 42 days (milligrams of N per 100 gm. of soil)	Quantity of nitrates present after 61 days (milligrams of N per 100 gm. of soil)
Unfertilized	Untreated		0.87	1.79	2.00
Do	do		.68	1.49	2.48
Average			.78	1.64	2.24
Unfertilized	Cyanamid	21	.43	.58	.58
Do	do	21	.49	.50	.58
Average			.46	.54	.58
Unfertilized	Ammonium sulphate.	21	1.10	4.33	6.93
Do	do	21	1.08	4.00	7.43
Average			1.09	4.17	7.18
Cyanamid, NH_3, 80 pounds; acid phosphate, P_2O_5, 80 pounds; potash, K_2O, 40 pounds.	Untreated		3.36	5.20	6.93
Do	do		2.89	4.84	7.43
Average			3.13	5.02	7.18
Cyanamid, NH_3, 80 pounds; acid phosphate, P_2O_5, 80 pounds; potash, K_2O, 40 pounds.	Cyanamid	21	.42	.95	6.12
Do	do	21	.49	1.04	7.17
Average			.46	.99	6.64
Cyanamid, NH_3, 80 pounds; acid phosphate, P_2O_5, 80 pounds; potash, K_2O, 40 pounds.	Ammonium sulphate.	21	3.36	10.40	18.57
Do	do	21	3.72	10.40	20.00
Average			3.54	10.40	19.28

It will be observed that the addition of cyanamid to the soil taken from the unfertilized portion of the field practically stopped nitrification and that even after a period of 61 days the nitrate content was not so great as in the untreated soil. Under exactly the same conditions the soil receiving ammonium sulphate showed a gradual increase in nitrate content as the incubation period increased. These results were obtained in spite of the fact that cyanamid is an alkaline material and ammonium sulphate an acid salt. Undoubtedly, if calcium carbonate had been added to the soil, a much higher percentage conversion of the nitrogen in both cyanamid and ammonium sulphate, particularly in the latter, would have been obtained.

For the sake of comparison soil was taken from an adjoining plot which for three successive years had received a complete fertilizer in which cyanamid was the nitrogen carrier. Samples of this soil were incubated in the laboratory either without additional fertilization or with cyanamid or ammonium sulphate. The results of these tests were somewhat similar to those obtained with soil from the unfertilized portion of the field, except that the nitrate content of soils and percentage nitrification of added materials was higher in practically every case. This may be attributed to a variety of factors, but undoubtedly the presence of an abundance of available phosphoric acid and potash was the most important factor. Furthermore, this well-fertilized soil probably had a more vigorous nitrifying flora, was less acid because of the field applications of cyanamid, and of course contained more nitrogen. Again, the marked retarding effect of cyanamid on nitrification is brought out. After 42 days the nitrate content of the cyanamid-treated soil was less than one-fifth of that of the control soil, and at the end of the experiment it was still not equal to that of the control. The nitrifying power of the soil was good, however, as shown by the results with ammonium sulphate.

RESULTS WITH SOILS FROM FIELD NO. 3

This soil was a much heavier loam than that of field No. 1, more compact, located at a lower elevation, and not as well drained as is desirable. The natural fertility was good and the yields of corn above the average, even without fertilization, but the results with cotton were very much poorer than on field No. 1. The nitrification results on soil from a fertilized and unfertilized portion of the field are given in Table 2.

TABLE 2.—*Nitrate content of soils from field No. 3 after fertilization with cyanamid and ammonium sulphate*

Treatment in field	Treatment in laboratory	Number of milligrams of nitrogen used per 100 gm. of soil	Quantity of nitrates present after 25 days (milligrams of N per 100 gm. of soil)	Quantity of nitrates present after 42 days (milligrams of N per 100 gm. of soil)	Quantity of nitrates present after 61 days (milligrams of N per 100 gm. of soil)
Unfertilized	Untreated		0.61	1.30	1.63
Do	do		.61	.96	1.86
Average			.61	1.13	1.74
Unfertilized	Cyanamid	21	.26	.34	.47
Do	do	21	.19	.30	.52
Average			.23	.32	.49
Unfertilized	Ammonium sulphate.	21	1.58	4.16	9.18
Do	do	21	1.37	4.00	8.67
Average			1.47	4.08	8.92
Cyanamid, NH_3, 80 pounds; basic slag, P_2O_5 80 pounds; potash, K_2O, 40 pounds.	Untreated		1.79	3.06	4.33
Do	do		1.86	2.74	4.16
Average			1.82	2.90	4.24
Cyanamid, NH_3, 80 pounds; basic slag, P_2O_5, 80 pounds; potash, K_2O, 40 pounds.	Cyanamid	21	.52	1.44	2.54
Do	do	21	.58	.80	2.48
Average			.55	1.12	2.51
Cyanamid, NH_3, 80 pounds; basic slag, P_2O_5, 80 pounds; potash, K_2O, 40 pounds.	Ammonium sulphate.	21	5.07	10.40	13.68
Do	do	21	4.00	10.00	14.05
Average			4.54	10.20	13.86

The general trend of these results conforms very closely to that for field No. 1. Again, cyanamid failed to nitrify to any marked extent during the duration of the experiment, and in every case in which this material was used there was less nitrate present at the end of two months than in the untreated soils.

Under the same conditions, ammonium sulphate nitrogen was converted into nitrates fairly rapidly. The nitrate content of the soil taken from the plot receiving a complete fertilizer was considerably higher than the nitrate content of the soil taken from the unfertilized plot, as in the case of field No. 1.

RESULTS WITH SOILS FROM FIELD NO. 4

The soil of this field was quite poor, well-drained, and similar in physical properties to that of field No. 1. It produced very good yields of cotton and corn when properly fertilized. Without fertilization it yielded practically nothing. When cyanamid was used with the soil from field No. 4, cotton gave a fair yield, but the retarding period lasted for so long after planting that the fertilizer could not be considered as satisfactory for cotton under the conditions used. The nitrate contents of the variously treated soils, as shown by laboratory studies, are given in Table 3.

TABLE 3.—*Nitrate content of soils from field No. 4 after fertilization with cyanamid and ammonium sulphate*

Treatment in field	Treatment in laboratory	Number of milligrams of nitrogen used per 100 gm. of soil	Quantity of nitrates present after 25 days (milligrams of N per 100 gm. of soil)	Quantity of nitrates present after 42 days (milligrams of N per 100 gm. of soil)	Quantity of nitrates present after 61 days (milligrams of N per 100 gm. of soil)
Unfertilized	Untreated		0.36	0.33	0.68
Do	do		.27	.33	.76
Average			.32	.33	.72
Unfertilized	Cyanamid	21	.10	.08	.12
Do	do	21	.08	.06	.13
Average			.09	.07	.12
Unfertilized	Ammonium sulphate.	21	1.04	2.00	3.71
Do	do	21	1.00	1.49	4.16
Average			1.02	1.74	3.93
Cyanamid NH_2, 80 pounds; basic slag, P_2O_5, 80 pounds; potash, K_2O, 40 pounds.	Untreated		2.67	3.59	6.93
Do	do		3.15	3.59	6.71
Average			2.91	3.59	6.82
Cyanamid, NH_2, 80 pounds; basic slag, P_2O_5, 80 pounds; potash, K_2O, 40 pounds.	Cyanamid	21	.65	.95	1.49
Do	do	21	.52	.87	1.53
Average			.59	.91	1.51
Cyanamid, NH_2, 80 pounds; basic slag, P_2O_5, 80 pounds; potash, K_2O, 40 pounds.	Ammonium sulphate.	21	12.68	20.80	27.37
Do	do	21	13.33	19.26	28.89
Average			13.01	20.03	28.13

The results obtained in field No. 4 are practically a repetition of the results obtained in fields Nos. 1 and 3 as given in Tables 1 and 2, respectively. Without fertilization, either in the field or laboratory, the nitrate content of this soil is exceedingly small, and the nitrifying efficiency very low. Even where ammonium sulphate was added in the laboratory to soil from the unfertilized field plot, only about one-seventh of the nitrogen has been converted into nitrates at the end of two months. Under the same conditions, soil from the fertilized plot showed a 100 per cent conversion of the ammonium sulphate nitrogen to nitrates. The use of cyanamid on both the fertilized and unfertilized soils, practically stopped all nitrification during the period of the experiment. The nitrate content in such cases was never as great as on the check plots.

DISCUSSION

The most striking point brought out by the results of the experiments is the fact that in all cases where cyanamid was applied to a soil in the laboratory, the amount of nitrates present in that soil was decreased below the checks, even in the case of the longest incubation

period. It will be noticed further that the use of cyanamid in the field over a period of two or three years resulted in a slight increase in the accumulation of nitrates. When additional quantities of cyanamid were added to these soils in the laboratory, the nitrates were markedly decreased and even after an incubation period of two months the supply was in no case as great as in the same soil kept in the laboratory to which no additional cyanamid was added.

The nitrification of ammonium sulphate in the soils which had received no field applications of fertilizer was rather slight, but in all cases the nitrate accumulation was greater than in the control plots or in those which received laboratory applications of cyanamid. The nitrification of ammonium sulphate was much more rapid and complete on the soils which had received field applications of cyanamid, phosphate, and potash made for the previous two or three years. The application of the complete fertilizer, together with the lime in the cyanamid, greatly increased the nitrifying capacity of the soils.

In considering the results reported here, it is realized that the laboratory applications of the cyanamid were far in excess of those used in the field. However, they were no greater than the applications commonly used in nitrification experiments. It should be remembered also that the cyanamid was thoroughly mixed with the soil samples in the laboratory, while in the field it is commonly drilled in the row. Regardless of the rate of application, the results are certainly comparable with those obtained with ammonium sulphate and give some indication of what takes place in many instances under practical conditions.

In the field experiments with the same soils, cyanamid could not be considered as a satisfactory fertilizer for cotton. In the presence of this material, growth was slow during the first two months but usually improved to a considerable extent later. The color of the plants was always an exceedingly dark green, indicating that they were being limited largely to ammonia and organic forms of nitrogen. The nitrification results with these same soils show that the supply of nitrates is actually less during the period of two months subsequent to fertilization with cyanamid than on the corresponding check plots. Since cotton requires nitrate nitrogen for its best growth, there seems to be little doubt that its failure to form readily from cyanamid largely explains why poorer results were usually obtained with this material than with any other source of nitrogen.

SUMMARY

Experiments are reported which show that cyanamid greatly retards nitrification in soils, thus explaining partially, at least, why in previous experiments cyanamid was not a satisfactory fertilizer for cotton. These experiments with soil samples taken from the field where the cotton was growing show that this crop had a smaller nitrate supply available when fertilized with cyanamid than did the cotton on the control plots. Doubtless the presence of certain injurious decomposition products of the cyanamid play a part also. These decomposition products act in some cases as direct plant poisons, and in others merely as poisons for the nitrifying bacteria.

EFFECT OF PUMPING FROM DEEP WELLS ON THE GROUND-WATER TABLE[1]

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INTRODUCTION

The increase of pumping in the San Joaquin Valley of California has focused attention on the need for information in regard to its effect on the ground-water supply.

Pumping in the San Joaquin Valley has a twofold purpose: (1) To supply water for irrigation and (2) to lower the water table and provide drainage. Although most of the water obtained as the result of drainage operations is used for irrigation, such economic use is only incidental to the primary purpose of the drainage pumps.

The pumping plant from which the data presented in this paper were obtained was installed in June, 1924, at Kearney Park, Calif.,[2] for the purpose of lowering the water table and providing drainage.

EQUIPMENT

PUMP

The pumping equipment consists of a 7-inch horizontal centrifugal pump, direct connected to a 20-horsepower motor having a speed of 1,160 revolutions per minute, and set in a 7-foot concrete pit. The pump operates under a total head of 25.4 feet and discharges about 1,550 gallons per minute. The pump and pit are covered with a fireproof metal house (fig. 1). The discharge is through a concrete pipe line, either to standpipes for irrigation, or into an open ditch where the water is wasted. Figure 2 shows the water flowing from the pipe line into the open ditch.

WELL

The well is 16 inches in diameter and when bored was 69 feet deep. Figure 3 shows the nature of the material encountered in the well. The quicksand at the bottom extends to an undetermined depth. The casing extends to a depth of 53 feet below the ground surface, where it is "landed" on a 10-foot clay stratum. In "developing" the well a large quantity of sand was pumped out so that there is probably a cavity of considerable size below the clay stratum.

The total depth of 69 feet is somewhat less than the usual depth of wells for the San Joaquin Valley,[3] but because of the quicksand it was impossible to go deeper without extending the casing or changing

[1] Received for publication Jan. 4, 1927; issued May, 1927.

[2] WEIR, W. W. PRELIMINARY REPORT ON KEARNEY VINEYARD EXPERIMENTAL DRAIN. Calif. Agr. Expt. Sta. Bul. 273, p. 103-123, illus. 1916.

KELLEY, W. P. THE PRESENT STATUS OF ALKALI. Calif. Agr. Expt. Sta. Circ. 219, 10 p. 1920.

[3] WEIR, W. W. PUMPING FOR DRAINAGE IN THE SAN JOAQUIN VALLEY, CALIF. Calif. Agr. Expt. Sta. Bul. 382, 38 p., illus. 1925.

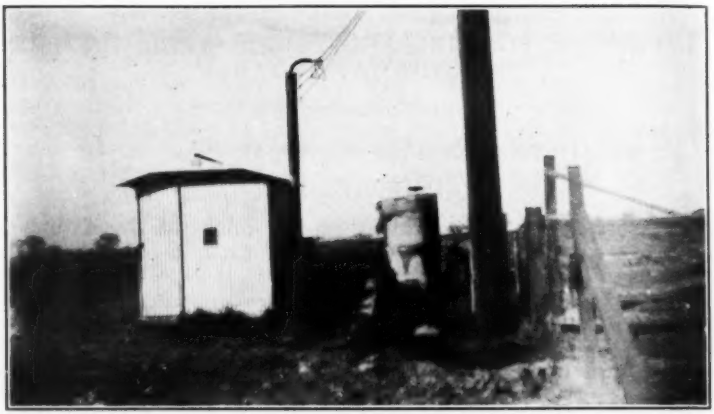

FIG. 1.—Pump house at Kearney Park, Calif. Test we No. 1 is located at the fence back o the power pole

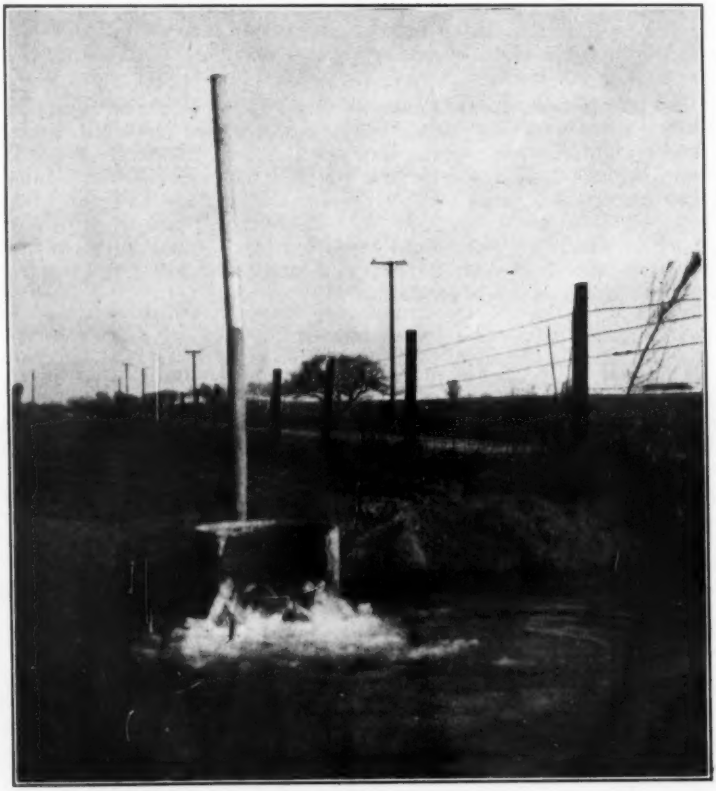

FIG. 2.—Waste pipe from the pump, showing the water entering the waste ditch

the type of casing in common use. In planning this study it was decided that a well having a capacity of 1,200 gallons per minute would be satisfactory. The actual discharge of 1,550 gallons per minute has amply justified the comparatively shallow well. This well, however, is similar in its general characteristics to the deeper wells of the valley. Its discharge could doubtless be increased by the installation of a larger pump, without either exhausting the well or creating an uneconomical draw down.

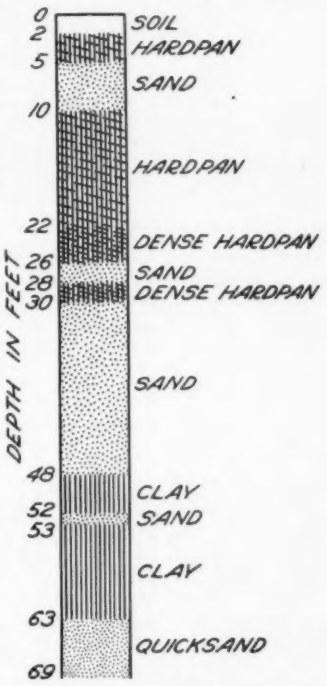

FIG. 3.—Nature of soil types encountered in drilling well at Kearney Park, Calif.

TEST WELLS

Twelve test or observation wells about 25 feet in depth were installed in lines radiating from the pump in the four cardinal directions. Figure 4 shows the locations of the test wells in relation to the pump. No. 1 is located just outside the pump house; Nos. 2, 4, 7, and 10 are 300 feet, Nos. 3, 5, and 11 are 1,000 feet, No. 8 is 1,300 feet, Nos. 6 and 12 are 2,000 feet, and No. 9 is 2,300 feet distant from the pump house. The test wells were put down with a small well-drilling outfit and are cased with 2-inch black iron pipe. The elevations in feet above sea level of the top of the casing of these wells were as follows:

No. 1, 252.70; No. 2, 252.19; No. 3, 254.02; No. 4, 252.35; No. 5, 254.96; No. 6, 253.56; No. 7, 253.48; No. 8, 250.85; No. 9, 249.91; No. 10, 252.58; No. 11, 253.58; No. 12, 256.04.

ANALYSIS OF TEST-WELL DATA

The depth to water in each of these wells was measured weekly from August 29, 1924. The test-well records for 1925 cover a complete cycle of rise and fall in the water table. As in previous findings in this locality,[4] the water table is lowest about the first of the year and highest in June. In Table 1 will be found the test-well data for the year 1925.

[4] WEIR, W. W. GROUND WATER FLUCTUATIONS AT KEARNEY PARK, CALIF. Hilgardia 1: 133-144, illus. 1925.

TABLE 1.—*Depth* [a] *to water in test wells, Kearney Park, Calif., 1925*

Date	Depth in feet to water in well No.—												Remarks
	1	2	3	4	5	6	7	8	9	10	11	12	
1925													
Jan. 2	12.9	12.3	13.0	12.8	15.9	15.4	13.0	12.3	12.2	12.7	13.4	15.3	Pump not running.
Jan. 9	12.9	12.2	13.5	12.8	15.9	15.2	13.0	12.2	12.1	12.7	13.4	15.3	Do.
Jan. 16	13.1	12.4	13.8	13.0	16.1	15.3	13.1	12.2	12.0	12.9	13.4	15.3	Do.
Jan. 23	13.1	12.5	13.9	13.0	16.1	15.4	13.1	12.2	11.9	12.8	13.4	15.3	Do.
Jan. 30	13.2	12.6	13.9	13.1	16.2	15.5	13.2	12.2	11.9	12.9	13.4	15.3	Do.
Feb. 7	13.0	12.4	13.4	12.8	16.0	15.4	13.0	12.1	11.9	12.7	13.3	15.1	Do.
Feb. 16	12.6	12.0	12.8	12.5	15.6	15.0	12.6	11.8	11.6	12.5	13.0	15.0	Do.
Feb. 23	12.2	11.5	12.0	12.0	15.2	14.7	12.2	11.5	11.5	12.0	12.5	14.5	Do.
Mar. 4	13.6	10.5	11.4	11.6	14.1	14.0	12.6	10.6	10.8	12.5	12.0	14.0	3 hours' pumping at time of reading.
Mar. 9	11.0	10.5	11.0	10.8	13.8	13.5	11.0	9.7	----	10.8	11.3	13.5	Irrigation around 9.
Mar. 16	10.9	10.4	11.4	10.8	13.7	13.3	10.9	10.0	9.4	10.7	11.2	13.2	
Mar. 23	16.8	13.6	12.4	14.4	13.8	13.2	15.2	10.6	9.6	15.2	13.0	13.5	Pump started Feb. 20, 1925.
Mar. 30	15.1	11.9	11.9	13.2	13.9	13.2	13.7	10.5	9.7	13.7	12.2	13.3	Pump off Mar. 28 and 29.
Apr. 6	15.5	12.2	10.5	12.9	----	11.1	14.1	9.8	9.3	14.1	12.0	12.9	Irrigation around 9.
Apr. 13	14.3	11.6	7.8	11.5	10.1	10.4	13.0	8.7	8.8	13.1	11.0	12.2	Irrigation around 2, 3, 4, 5.
Apr. 20	13.7	11.4	9.3	11.3	11.3	10.1	12.8	8.3	7.5	13.0	11.0	11.8	
Apr. 27	14.2	11.0	9.4	11.7	11.6	10.1	12.9	7.8	6.3	13.0	10.8	11.7	
May 11	14.0	11.1	7.7	11.2	10.2	8.4	12.5	7.2	5.3	12.6	10.6	11.3	Irrigation around 3 and 6. Pump stopped May 9.
May 18	9.6	----	6.4	----	9.1	7.8	9.4	5.8	5.1	9.1	8.5	10.6	Irrigation around 2 and 4.
May 25	11.1	8.8	6.9	8.1	7.5	7.1	10.2	5.3	4.6	10.6	9.1	10.4	Pumping daytime only, May 14 to 21.
June 1	11.8	8.9	6.6	8.8	8.6	7.6	10.6	5.7	4.7	10.7	9.1	10.3	
June 8	10.7	9.6	7.5	8.6	8.6	7.4	10.3	5.7	4.3	10.9	9.3	10.4	
June 15	11.5	8.7	7.4	9.3	9.4	8.1	10.2	5.7	4.2	10.4	9.1	10.5	Pump stopped June 14 for 1 day.
June 22	12.8	10.1	7.4	9.8	9.4	7.8	11.2	5.5	3.8	11.5	9.5	10.4	
June 29	13.4	10.8	7.9	10.7	10.4	8.6	11.9	6.4	4.3	12.1	10.0	10.8	
July 6	13.9	11.2	9.2	11.1	11.2	9.4	12.5	7.6	----	12.6	10.4	11.0	Irrigation around 9.
July 13	14.6	11.8	10.4	11.8	12.0	10.1	13.0	7.4	4.9	13.1	10.9	11.5	Do.
July 20	12.1	9.2	8.4	9.9	11.6	10.4	11.2	7.5	5.8	11.2	10.3	11.8	No explanation.
July 27	14.5	11.7	9.3	11.6	12.1	10.5	13.1	8.2	6.4	12.1	11.1	11.9	
Aug. 3	14.8	12.0	11.1	12.4	12.7	11.0	13.4	9.1	6.2	13.6	11.6	12.4	
Aug. 10	15.5	13.3	12.4	13.4	13.7	11.8	14.2	9.0	7.1	13.8	12.3	12.9	
Aug. 17	10.3	9.7	9.9	9.7	12.5	11.8	10.1	8.8	7.6	10.1	10.9	12.7	Pump stopped Aug. 14–17, inclusive.
Aug. 24	16.5	13.4	12.9	14.0	14.1	12.4	14.9	9.7	8.0	15.0	12.9	13.4	
Aug. 31	16.3	14.2	13.6	14.3	14.9	13.1	15.0	10.2	8.5	15.1	13.3	13.8	
Sept. 7	16.6	13.9	12.3	13.7	14.6	13.2	15.2	10.4	8.9	15.5	13.5	14.1	Irrigation close to 2, 3, 4, and 5.
Sept. 14	16.0	13.2	11.3	12.7	13.5	12.6	14.6	10.0	8.9	14.9	13.0	13.9	Water in Houghton.
Sept. 21	17.0	14.0	13.1	14.4	14.6	13.0	15.4	10.2	8.6	15.5	13.4	14.0	Houghton nearly dry.
Sept. 28	17.5	14.5	14.0	15.2	15.4	13.6	15.9	10.7	8.9	16.0	13.9	14.4	Houghton dry.
Oct. 5	15.2	14.5	14.3	14.2	15.7	14.1	15.5	10.8	9.1	15.0	13.9	14.6	Pump off 7 hours.
Oct. 12	12.7	12.2	13.4	12.4	15.1	14.0	12.6	10.7	9.3	12.4	12.8	14.4	Pump shut off Oct. 9, 1925.
Oct. 19	14.7	13.4	13.9	13.8	15.5	14.1	14.7	11.0	9.7	14.3	13.5	14.4	Pump run 40 hours, Oct. 10 to 19.
Oct. 26	14.9	11.9	13.2	13.3	15.0	14.0	13.6	10.8	9.8	13.6	12.8	14.4	Pump run 7½ hours during week.
Nov. 2	14.3	12.4	13.7	13.5	15.4	14.2	14.2	11.0	10.0	13.9	13.2	14.4	Pump run 12 hours.
Nov. 9	12.3	11.8	13.2	12.2	15.2	14.3	12.2	11.0	10.2	12.0	12.6	14.5	No pumping.
Nov. 16	12.4	11.9	13.3	12.2	15.2	14.4	12.3	11.1	10.4	12.1	12.7	14.6	Do.
Nov. 23	12.3	11.9	13.3	12.2	15.3	14.5	12.2	11.1	10.4	12.1	12.7	14.6	Do.
Dec. 2	12.3	11.8	13.3	12.2	15.2	14.4	12.3	11.2	10.6	12.1	12.7	14.5	Rain.
Dec. 7	12.3	11.8	13.3	12.2	15.2	14.4	12.3	11.1	10.6	12.0	12.6	14.5	No pumping.
Dec. 14	12.4	11.8	13.3	12.2	15.2	14.4	12.3	11.2	10.6	12.1	12.6	14.5	Do.
Dec. 21	12.4	11.8	13.3	12.2	15.2	14.4	12.3	11.2	10.7	12.1	12.6	14.5	Do.
Dec. 28	12.3	11.8	13.3	12.2	15.2	14.5	12.2	11.1	10.7	12.1	12.7	14.5	Do.

[a] Depth in feet below top of well casing. Top of casing 1 foot above ground surface.

In Figure 5 is shown the position of the water table in relation to the ground surface, on three days in 1924. On June 4 the water table was practically a straight line through both the north and south and the east and west wells. This was the highest point recorded for that year. Measurements of the water table were taken before any pumping was done and therefore represent the normal position for that day. There is a slight slope from east to west (shown more clearly in Figures 6 and 7), and also from north

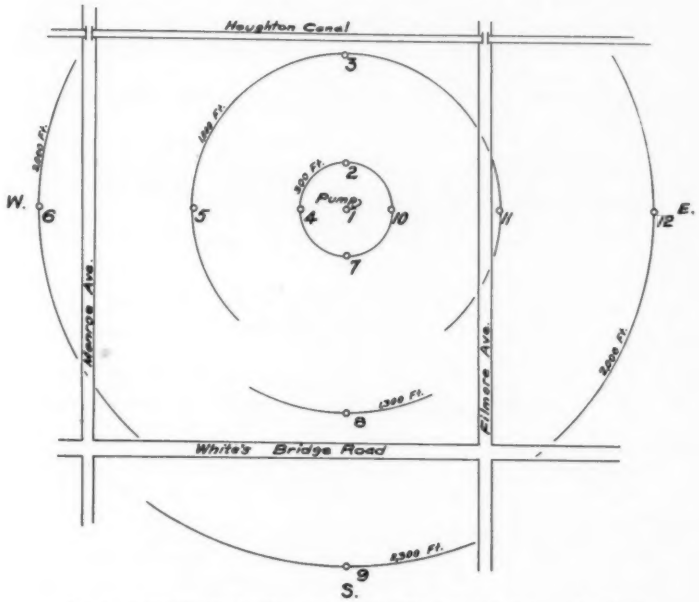

FIG. 4.—Sketch showing locations of test wells with reference to the pumping plant

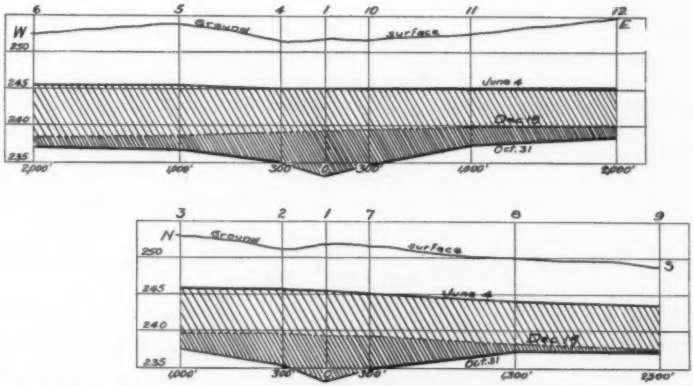

FIG. 5.—Highest and lowest water table in 1924. On June 4 the pumping had not yet started. October 31 was the end of the pumping season and December 19 the date of the lowest water during the period when the pump was not in operation

to south. In 1924 the pumping season began August 29 and ended October 31. On the last-named date the water table stood as indicated in the diagram. On December 19, or about six weeks after the pumping season closed, the water table had reached its normal low point

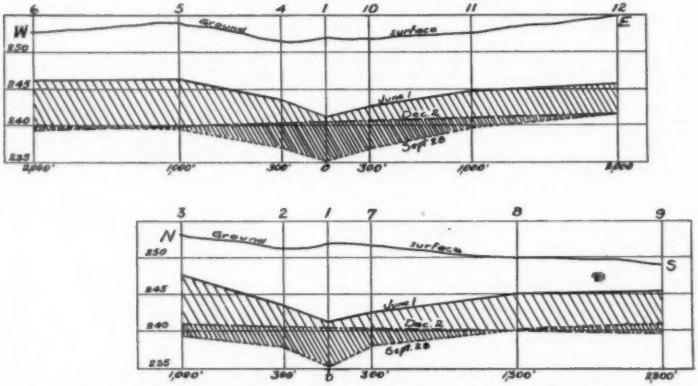

FIG. 6.—Highest and lowest water table June 1 and September 28, respectively, during the 1925 pumping season, and on December 2, several weeks after the close of the pumping season

for the winter, which, as will be observed, is above that of October 31. The lightly shaded area in Figure 5, indicating the change from June 4 to December 19, is due to the normal seasonal fluctuation and not to pumping.

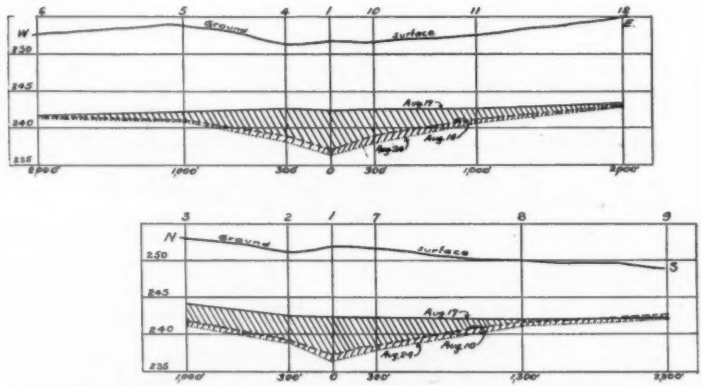

FIG. 7.—Graph shows the complete recovery of the water table in a brief period of three days during which there was no pumping

In 1925 the pump was started March 20 and was operated until October 26. There were, however, several intermissions, particularly during the early and late seasons. From May 14 to 21 the pump was operated during the daytime only. Figure 6 shows conditions for 1925 similar to those in Figure 5 for 1924. Although

by June 1, 1925, the pump had been in operation for several weeks, the water table had risen to the height indicated. The pump was operated only intermittently after September 28 and at that date the table had not reached the minimum depth.

Figure 7 shows the position of the water table on August 10, at which date the pump had been in continuous operation for several weeks. During the last three days of the following week the pump was not operated, with the result that the water table rose as indicated by the line "August 17" in Figure 7. This position is parallel to those shown in Figures 5 and 6 and is undoubtedly the normal position for that date. With the resumption of pumping for the week ending August 24, the water table again receded to a position parallel to that of August 10. This indicates that in as short a period as three days there is an entire recovery of the water table from the effects of previous pumping. The difference between the curves for August 10 and 24 is accounted for by the normal recession of the water at that time of the year.

The diagrams show that pumping has noticeably affected the water table at a distance of 2,000 feet from the pump. The normal slope of the water table toward the west and south is reversed in direction for a distance of 2,000 feet while the pump is in operation. Except for a few days in 1925, when the experimental fields were being heavily irrigated and pasture land around well No. 9 was being flooded, the tile drains with which this tract is supplied [5] contained no water, the pump having lowered the table below the tile.

In Figure 8 are shown the actual weekly fluctuations in the water table of the 12 test wells for the year 1925. Because of its proximity to the pump, well No. 1 is assumed to reflect all of the major changes in the operations of the pump. As the readings were taken only once a week, some of the minor fluctuations may be missing. It will be observed that wells Nos. 2, 4, 7, and 10, which are only 300 feet distant from the pump, show very nearly the same fluctuations; those 1,000 feet away are less affected; and those 2,000 feet away are only very slightly influenced. Although they are both 2,000 feet from the pump, wells Nos. 6 and 12 differ from each other considerably more than do wells Nos. 2 and 4, for instance, which are both 300 feet from the pump. This condition is due to other factors than the influence of the pump. Wells Nos. 6 and 12 are actually 4,000 feet apart, while wells Nos. 2 and 4 are only about 425 feet apart. Wells Nos. 6, 12, and 9 are located in areas which receive entirely different cultural and irrigation treatments. Well No. 6 is in an alfalfa field, well No. 12 is in a vineyard, and well No. 9 is in a Bermuda-grass pasture.

In the wells located 300 and 1,000 feet, respectively, from the pump there was a slight rise in the water table on October 26 that was not shown in well No. 1. The pump was operated intermittently at this season, and the readings may have been taken shortly after the pump had resumed operation and had had time to affect the water table only in the immediate vicinity.

It is interesting to observe that the final stopping of the pump in October did not affect the normal seasonal lowering in wells more

[5] WEIR, W. W. PRELIMINARY REPORT ON KEARNEY VINEYARD EXPERIMENTAL DRAIN. Calif. Agr. Expt. Sta. Bul. 273, p. 103–123, illus. 1916.

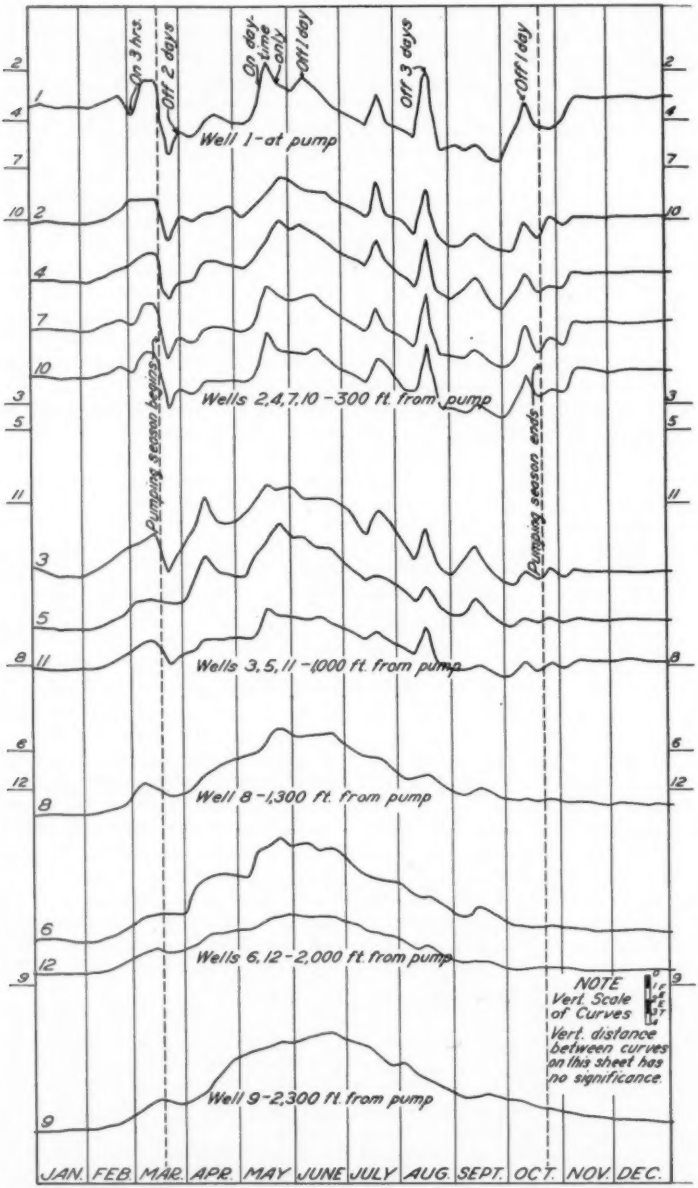
On 3 hrs.
Off 2 days
On day-time only
Off 1 day
Off 3 days
Off 1 day
Well 1-at pump
Wells 2,4,7,10-300 ft. from pump
Pumping season begins
Pumping season ends
Wells 3,5,11-1000 ft. from pump
Well 8-1,300 ft. from pump
Wells 6,12-2,000 ft. from pump
Well 9-2,300 ft. from pump
NOTE
Vert. Scale of Curves
Vert. distance between curves on this sheet has no significance.
JAN. FEB. MAR. APR. MAY JUNE JULY AUG. SEPT. OCT. NOV. DEC.

FIG. 8.—Rise and fall in the water table at different distances as the result of variations in the operation of the pump. Readings were taken weekly. Well No. 1 is assumed to reflect all of the major changes in pumping operation. Average ground surface at each well is indicated on the borders of the graph

than 1,000 feet from the pump, whereas in those within 1,000 feet of the pump there was an immediate rise to the normal position.

In order to bring out more clearly the variations in individual wells, all the curves shown in Figure 8 for any given distance are superimposed and shown in Figure 9. The water table as of January 1 is taken as a common point on each curve. The average of the

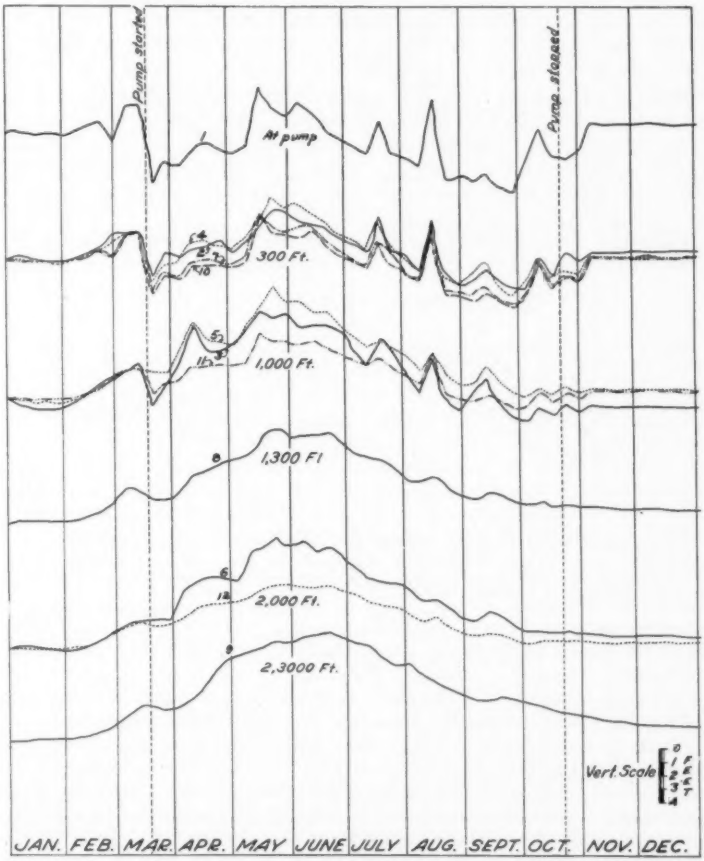

FIG. 9.—Water table for wells the same distance from the pump, superimposed. Water table as of January 1 taken as a common point

superimposed curves in Figure 9 is again superimposed in Figure 10 and shows plainly the effect of distance on the influence of the pump. Here again the water table as of January 1 is taken as a common point for all distances. The average curve for the 2,000-foot wells shown in Figure 9 is remarkably similar to the average water-table curve for the general region, as shown in Figure 5 of the writer's publication, "Ground Water Fluctuations at Kearney Park, Calif."[6]

[6] WEIR, W. W. GROUND WATER FLUCTUATIONS AT KEARNEY PARK, CALIF. Hilgardia 1:133–144, fig. 5. 1925.

Although no continuous measurements were recorded covering the discharge from this pump, a close approximation of the amount of water discharged during the year may be obtained by comparing the rating given the pump shortly after installation, with the record of the number of days it was in operation. From March 20 to October 26, a period of 210 days, the pump was in operation approximately 197 twenty-four-hour days, with an average discharge of over 1,500 gallons per minute or 1,182 acre feet for the season.

SUMMARY

The data obtained from the operation of the pump herein described is believed to be representative of that which might be obtained over a considerable portion of the east side of the San Joaquin Valley where substrata conditions are similar to those at Kearney Park. The conclusions enumerated below are applicable generally to pumps of the capacity of the one used.

Effective drainage can be obtained for a distance of more than 1,000 feet from the pump. (The writer has shown in a previous

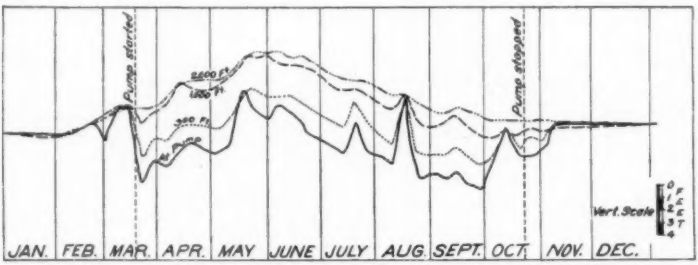

FIG. 10.—Average curves for test wells at varying distances from pump, superimposed. Water table as of January 1 taken as a common point. Each curve is the average of a group of curves (fig. 9) for the given distance

paper[7] that pumping from shallow surface wells or sumps does not affect the water table to an appreciable extent beyond a distance of 100 feet.)

In order to maintain a constant depth of water table, it is necessary to keep the pump in continuous operation during the pumping season.

There is no indication that this pump has had any permanent effect on the amount of water available for pumping. Undoubtedly, however, a large number of pumps operated continuously would permanently lower the water table, even though there was no diminution in the amount of water supplying the underground reservoirs.

There is a rapid movement of water through the water-bearing sands and gravels of the region, as evidenced by the rapid recovery of the water table to normal when the pump is stopped.

For increased effectiveness in lowering the water table and thereby providing drainage, and in supplying water for irrigation, it is likely that pumps of larger capacity than the one used in the work outlined in this discussion may be installed without either creating an uneconomical lift or bringing about a significant diminution of the water supply.

[7] WEIR, W. W. EFFECT OF PUMPING FROM A SHALLOW WELL ON THE GROUND WATER TABLE. Jour. Agr. Research 11:339-357, illus. 1917.

RECIPROCAL EFFECTS FROM GRAFTING[1]

By FRED W. HOFMANN

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INTRODUCTION

It has long been recognized that grafting can effect appreciable changes in the component parts of a plant. Commenting on these changes, Jost (*2*)[3] says, "As Strasburger has found plasma bridges between stock and scion, a migration of plasma particles is not impossible." Since striking modifications in the morphology as well as in the physiology of the affected parts occur, the field for further inquiry seemed so promising as to persuade the writer to make further investigations along this line. For this purpose various reciprocal grafts of beans of different varieties and even of different species were made. The experimental work was done at the University of Illinois.

MODIFICATION OF STOCK

The extent to which grafting modifies the rootstock has been noted by several workers. Lindemuth (*3*) describes a graft of Abutilon top-worked with an annual plant, *Modiola carolina*, which was kept for three years and five months. Sahut (*8*) refers to various cases of evergreen scions, such as *Crataegus glabra* and Raphiolipsis, being grafted on the common quince, which is deciduous. He also describes a graft of the late-opening St. Jean walnut, top-worked on the common walnut, which resulted in holding back the stock for over a month. An evergreen cherry, Laurier-Amands, top-worked with a deciduous variety which was kept as long as the ungrafted deciduous stock, was also mentioned, together with early-leafing American varieties which were correspondingly retarded when top-grafted with scions of late-leafing European sorts. An extensive survey made by Vard (*9*), following the severe winter of 1890–91 in France, showed that normally hardy stocks top-worked with scions of tender varieties, such as the tea and Bourbon roses, were killed; and in no instance were such top-worked stocks as hardy as the ungrafted hardy types. Webber et al. (*5*), in observations on the freezing winter of 1913 in California, refer to the definite influence of the tops upon the stock, in grafts of citrus varieties. Not only were the tender lemon tops killed but the injury from freezing extended 3 to 4 inches down the normally hardy stock. Pomello seedlings when grafted with scions of the tender varieties were likewise injured, while the ungrafted stocks were scarcely touched.

[1] Received for publication Nov. 12, 1926; issued May, 1927. Contribution from the Division of Plant Breeding, Department of Agronomy, Agricultural Experiment Station, University of Illinois. Published with the approval of the director of the station.

[2] Now associate horticulturist, Virginia Agricultural Experiment Station.

[3] Reference is made by number (italic) to "Literature cited," p. 676.

METHOD OF MAKING GRAFTS

Beans are very tender and can be grafted only while they are growing on their own roots. For this reason the approach method of grafting was found to be the most effective. The plants to be grafted were grown close together in a gallon pot. After the seedlings had straightened out, or prior to the time that the first whorl of leaves had formed, each seedling was wounded or abrased up and down the length of the stem. The two surfaces were pressed together and tied securely. To hasten union between the tissues, numerous wounds were made by piercing both stems all the way through with a needle. The callus growth which resulted made a very effective coalescence of the component tissues. After the union was established the roots corresponding to the scion were severed, thus leaving a rootstock of one variety, top-grafted with a scion of another.

LONGEVITY OF STOCK

Normally under field conditions *Phaseolus vulgaris*, the navy bean, is an annual, living about three or four months. Even where protected from freezing and maintained under the most favorable greenhouse conditions, it seldom lives more than four to five months. Under field conditions, *P. lunatus*, the Lima bean, may be kept growing until it is killed by frost. Under greenhouse and semitropical conditions, with protection from freezing, Lima bean plants can be made to live for several years. The writer has kept some for over three years in a greenhouse at the University of Illinois.

Reciprocal grafts were made between the two species for the purpose of studying the effect upon the stock. Very noticeable differences were secured with such grafts. Lima rootstocks top-worked with navy scions produced plants which lived no longer than nongrafted navy bean plants. However, by top-working navy bean rootstocks with Lima bean scions, plants were produced which had the longevity of nongrafted Lima bean plants. This shows the marked degree to which the rootstock may sometimes be modified when the plant is top-worked.

NATURE OF STARCH GRAINS IN THE STOCK

Several studies were made of the starch to determine as far as possible what changes might have occurred in the stocks of Lima and navy bean grafts. Two questions naturally arose: (1) To what extent is the elaborated material produced in the scion translocated to the rootstock, and (2) is the elaborated material modified before, during, or after such translocation?

In these studies the differences between the starch grains of the Lima and the navy bean, as brought out in Reichert's (*6*) tests, were carefully observed. In this way an effort was made to compare the starch grains of the top-worked stocks and the nongrafted plants of the two species. The differences between the starch grains were so extremely minute, however, as not to allow a definite conclusion to be drawn. Nevertheless, it seems reasonable to believe that the elaborated materials produced in the scion of a species that is quite different from the rootstock should become altered to some extent when translocated to the rootstock.

ANTHOCYANIN TRANSFER

Meyer and Schmidt (*4*) have shown that a soluble alkaloid such as nicotine will pass freely from a tobacco scion to a potato rootstock. In the case of glucosides, however, Guignard (*1*) states that there is no passage from one graft symbiont to the other. Since anthocyanin is a soluble glucoside, studies were made to determine whether or not this pigment was transferred from the tissue of one graft component to that of the other, and, if so, to what extent such a union affected either graft component.

The navy bean plants used in these experiments had a clear, bright green color and were entirely free from any anthocyanin pigment. In decided contrast, the plants of Refugee bean strain selected for the experiment were characterized by a very dark, reddish purple anthocyanin pigment.

Reciprocal grafts between the pigmented and the nonpigmented varieties showed no incompatibility whatever, as they produced well-established unions. In spite of this fact, however, the sharp line of color of the anthocyanin pigment showed where the two tissues met. Furthermore, throughout the entire life of the grafts, no indication was present of a passage of the anthocyanin pigment from the Refugee tissue to that of its graft component. Nor was there any diminution of color in the pigmented graft component. From these results it can be definitely concluded that there was no passage of the dark-colored anthocyanin from the one graft component to the other, and also that neither graft component was in any way modified in so far as pigment was concerned.

ALTERED BACTERIAL SYMBIOTIC SPECIFICITY

Another difference between the navy and the Lima bean is in respect to their specificity with a particular strain of the symbiotic-forming bacteria, namely, *Bacillus radicicola*. Normally, the strain of bacteria that is specific to one of the bean species will not inoculate the other. Studies were therefore made to determine to what extent reciprocal grafting of Lima and navy beans modifies this specificity.

Seeds of reciprocal grafts were planted and the roots of the resulting seedlings were inoculated. The offspring of the navy bean scions, top-grafted on Lima, were inoculated with Lima bean bacteria, and the offspring of the Lima bean scions, top-grafted on navy, were inoculated with navy bean bacteria. Examination of the roots showed nodules in both cases, indicating that cross inoculation was successful.

From this experiment it is evident that cross inoculation was effected with the seedlings coming from scions that had been top-grafted to the particular species to which the bacteria were specific. Thus grafting of Lima on navy beans, and vice versa, altered the specificity of the seed produced on the scion. A fuller report on this work is given by T. E. Richmond (*7*), who at the time of these experiments was a member of the division of soil biology, department of agronomy, University of Illinois, and made the inoculations on this material.

SUMMARY

The results of the investigation herein reported may be summarized as follows: (1) Reciprocal grafts can be made between *Phaseolus vulgaris* and *P. lunatus;* (2) the longevity of the rootstock of *P. vulgaris* may become materially altered when *P. vulgaris* is top-grafted with *P. lunatus;* (3) although anthocyanin is described as a soluble glucoside, no passage of this pigment was observable in the graft symbionts between plants of anthocyanin and nonanthocyanin varieties; (4) seeds developed on reciprocal grafts of *P. vulgaris* and *P. lunatus* produced plants which could be cross inoculated, showing that an altered bacterial symbiotic specificity had been brought about.

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INFLUENCE OF FORM AND PROPORTION OF LIME USED AND OF METHOD OF MIXING ON THE RESULTING BORDEAUX MIXTURE[1]

By E. B. HOLLAND, *Research Professor of Chemistry*, and C. O. DUNBAR and G. M. GILLIGAN, *Investigators in Chemistry, Massachusetts Agricultural Experiment Station*

INTRODUCTION

Ever since Millardet (*8, 9, 10, 13, p. 734*)[2] noted that a mixture of copper sulphate and lime prevented downy mildew on grapevines in 1882, and recommended its application as a fungicide, the "copper mixture of Gironde" (*11*) has undergone numerous modifications in copper content, ratio of lime to copper sulphate, and method of preparation in the hands of different investigators. Yet the influence of the form and the proportion of lime, and of the method of mixing on the resulting Bordeaux (bouillie bordelaise) is not clearly understood and merits additional study. Space does not permit an adequate review of the literature pertaining to this subject but attention will be called to some of the earlier investigations.

REVIEW OF LITERATURE

According to Millardet and Gayon (*12*) and most contemporary writers, calcium hydroxide reacts with copper sulphate to form cupric hydroxide and gypsum; but Pickering (*1, p. 26, 27; 17, p. 1991, 1997, 2000; 18, p. 1852*) has shown that limewater added to a solution of copper sulphate precipitates a blue basic sulphate increasing in basicity toward equal mols, converted by a large excess into what is termed a double sulphate and, by still more lime, into a double oxide. The variance between the two views is essentially a difference as to the point at which the sulphate radical disappears from the resulting copper precipitate. Whether the copper precipitates are chemical entities or mixtures is difficult to determine, owing to their instability and the presence of insoluble by-products but, in the last two instances at least, the compounds designated are empirical. The reaction in more concentrated mixtures such as Bordeaux is evidently somewhat depressed. Freshly prepared copper precipitates, with the exception of the tribasic sulphate, are usually gelatinous, of a high degree of hydration, and of excellent suspension. The gypsum and excess lime in suspension substantially increase the bulk of the precipitate and the portion in solution increases the viscosity of the serum.

Experiments made from 1885 to 1890 by Ferrand (*3*), Millardet and Gayon (*14, p. 701; 15*), Gaillot (*4*), Patrigeon (*16, p. 701*), Viala and Ferrouillat (*19, p. 27*), Mach (*7*), and many others, led to a reduction in the amount of copper sulphate and more or less change in the proportion of lime. Mixtures with a smaller percentage of copper sulphate were found effective and, with a lower ratio of lime, more active and adherent. Slightly alkaline, neutral, and even acid

[1] Received for publication Dec. 2, 1926; issued May, 1927. From the Department of Chemistry, Massachusetts Agricultural Experiment Station; printed with the permission of the director of the station.

[2] Reference is made by number (italic) to "Literature cited," p. 685.

mixtures received attention. In this country there has been a tendency recently to revert to a higher ratio of lime with a view of preventing foliage injury.

INFLUENCE OF THE FORM AND PROPORTION OF LIME USED

The two ingredients employed in preparing Bordeaux mixture are copper sulphate and lime (calcium hydroxide), although supplementary products are occasionally added. The aim is to secure the highest possible dispersion of the resulting precipitate, as the physical properties are of paramount consideration. The copper sulphate employed in the experiments reported was of good quality and substantially free from other metals. The solution was standardized as to copper content in most instances.

FORMS OF LIME USED

Four distinct forms of calcium hydroxide were employed, i. e.: Limewater, chemically precipitated calcium hydroxide, milk of lime, and commercial hydrated lime. The limewater was prepared from diluted milk of lime by filtration, thus assuring a fairly definite amount of active calcium hydroxide, although the low content limits the concentration of the resulting spray. In the Massachusetts laboratory quickly prepared solutions contained only 0.113 gm. of CaO in 100 c. c., as determined against n/10 hydrochloric acid, using phenolphthalein as indicator. This is probably due to incomplete saturation, partial carbonation, or selective absorption of the filter paper, and possibly to all three.

The chemically precipitated calcium hydroxide was prepared by the reaction of sodium hydroxide on calcium chloride and dialyzed free from chlorides, although any other modification that assures a homogeneous suspension of fine, active particles, free from impurities, might be employed. Such a product is nearly ideal for the preparation of Bordeaux mixture but too expensive for practical use.

The milk of lime was prepared by carefully slaking a high-grade granulated "fat" lime. The objective is complete hydration with a high degree of dispersion and the formation of a smooth, creamlike product having good suspension and adhesive properties. One of the writers (Dunbar), on a trial and error basis, obtained excellent results by adding 3 volumes of granulated lime to 5 of water, mixing vigorously and gradually introducing additional water. Subsequently, 1 part of lime added to 1.6 volumes of water and diluted with boiling water was found more satisfactory. Coarse particles of lime in the milk, whether due to poor stock or method of slaking, reduce the efficiency of the resulting Bordeaux, since they serve as nuclei for the formation of larger aggregates when the lime is mixed with the copper sulphate. Straining the milk of lime through double thickness of cheesecloth was adopted as preferable to screening, but this requires more time.

As the principal difficulty in preparing Bordeaux mixture with milk of lime is in the proper slaking of the lime, commercial hydrated lime was considered as a substitute. It has undoubtedly been used by others but to what extent is difficult to say. Of the hydrated limes on the market, agricultural lime is unsuitable, but some brands of plasterers' or finishers' lime are fairly soft, bulky, and substantially free from grit; and the so-called "chemical" hydrated lime is of greater

purity and better physical characteristics. The sample of plasterers' lime employed had a volume of 2.4 c. c. per gram, 99 per cent passing a 0.5 mm. sieve, and contained 42 per cent of active lime, calculated as oxide. As dry hydrated lime disperses slowly in water it was allowed to soak overnight before it was used to prepare Bordeaux, as the suspension of the latter is materially increased thereby. Several samples of "chemical" hydrated lime received recently had an average volume of 2.6 c. c. per gram and contained 67.3 per cent of active lime (theoretical for total CaO, 75.682 per cent). The manufacturers claim 96.5 to 99 per cent will pass a 200-mesh screen.

By active lime is meant that portion which can be readily determined by titration against standard acid to the initial disappearance of color, using thymolphthalein as indicator, thereby excluding coarse particles that are slow to react. The active portion of precipitated lime and milk of lime was determined in a similar manner and all tests were based on the active and not on the total lime present. Inert material is objectionable from the standpoint of both application and spray residue.

With precipitated lime, milk of lime, and hydrated lime, the fineness and uniformity of the particles seem to determine in large measure the physical properties of the resulting Bordeaux mixture. The degree of dispersion of the lime in the three forms evidently decreases in the order named.

PROCEDURE AND RESULTS

A series of experiments with 12 different ratios of lime to copper sulphate was instituted to determine the relative activity of the four forms of calcium hydroxide and the suspension of the Bordeaux mixtures prepared with them. The same concentration of copper sulphate, equivalent to that of ordinary Bordeaux, 4–4–50, was employed except in the case of limewater where a like strength was impossible, but the ratios of lime to copper sulphate were the same. Rubber-stoppered, glass museum jars of the following dimensions were employed for the purpose. Total height, 305.5 mm. (14 inches); length and outside diameter of the body, 303.0 by 63.5 mm. (13 by 3.5 inches); and capacity to the neck, 850 to 900 c. c. The jars were graduated to 780 c. c. and the length of the column determined for each jar. Suspension is expressed in percentage. The results at the end of 1, 2, and 3 hours are reported in Table 1.

Seven hundred and fifty cubic centimeters of the limewater were added to 30 c. c. of solution containing the required amount of copper sulphate for the different ratios. In most other cases, 663 c. c. of solution containing 7.49 gm. of copper sulphate were added to 117 c. c. of lime in suspension, containing the necessary amount of active lime for the different ratios. With precipitated lime and milk of lime the demands of the two highest ratios necessitated increasing their volume to 200 and 250 c. c., respectively, and reducing the volume of copper sulphate correspondingly. In American practice the composition of Bordeaux mixture is usually designated by figures such as 4–4–50, which signifies 4 pounds of copper sulphate and 4 of quicklime in 50 gallons of mixture, and is substantially equivalent to 1 part (or 1 per cent) of each in 100. Some writers term such a mixture 1 per cent Bordeaux and indicate the proportion of lime by ratio 1 : 1. The former method is less confusing and is employed in the text. Owing to inability to control the reaction, considerable varia-

tion is inevitable in such tests. Average results therefore are reported, those tests that suffered appreciable decomposition being excluded, although by so doing a fictitious value is given those mixtures that were particularly susceptible.

TABLE 1.—*Ratio of volume of suspended Bordeaux to total volume of liquid, when different forms and proportions of calcium hydroxide were used*

No.	Bordeaux mixture	Limewater			Precipitated lime			Milk of lime			Hydrated lime		
		1 hr.	2 hrs.	3 hrs.	1 hr.	2 hrs.	3 hrs.	1 hr.	2 hrs.	3 hrs.	1 hr.	2 hrs.	3 hrs.
		P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
1	4–0.672–50	73.8	39.8	32.3	87.0	78.1	71.7	82.2	70.2	62.3	69.4	53.0	45.6
2	4–0.720–50	93.7	86.1	78.3	91.8	84.5	78.8	93.9	88.3	83.3	87.3	75.5	65.3
	Neutral:												
3	4–0.808–50	95.6	91.0	86.1	96.6	93.2	90.1	97.0	94.2	91.4	94.6	90.6	83.4
	Alkaline:												
4	4–1.000–50	92.2	84.6	77.1	97.2	94.6	92.5	97.1	94.6	91.7	95.3	91.5	87.5
5	4–1.500–50	78.8	52.3	40.6	97.6	95.4	93.3	97.3	94.7	91.9	96.0	92.1	88.7
	Basic:												
6	4–2.000–50	34.0	23.9	18.9	97.3	95.2	93.0	97.5	95.0	92.6	96.4	92.7	89.0
7	4–2.250–50	29.3	20.6	16.3	97.2	94.9	92.5	97.3	94.6	91.9	96.2	92.5	88.5
8	4–2.500–50	27.0	19.0	15.0	97.1	95.0	92.4	97.2	94.4	91.7	95.7	91.5	86.9
9	4–3.000–50	22.4	15.0	11.9	97.1	94.7	92.2	97.1	94.3	91.5	95.5	90.4	84.9
10	4–4.000–50	16.8	11.3	8.2	96.6	93.7	91.1	96.7	93.3	90.0	94.1	87.6	79.1
	Highly basic:												
11	4–6.000–50	12.5	8.0	6.2	92.1	87.3	83.4	95.1	90.7	87.0	88.1	76.9	68.2
12	4–8.000–50	9.4	6.6	4.9	89.1	83.5	79.7	94.3	90.4	86.6	84.2	72.2	63.8

With limewater the concentration of copper sulphate varied from 0.647 gm. in 100 c. c. in No. 1 to 0.054 gm. in 100 c. c. in No. 12. No. 1 gave a light blue, flocculent precipitate; No. 2 a fairly gelatinous, blue precipitate, and No. 3 a full blue, gelatinous precipitate with the highest suspension. In Nos. 4 to 12 the color and suspension gradually decreased. None of these mixtures decomposed within three hours.

With chemically precipatated calcium hydroxide the mixture 4–0.672–50 (No. 1) yielded a light blue, flocculent precipitate, increasing to a full blue, gelatinous precipitate in the mixtures 4–1–50 (No. 4) and 4–1.5–50 (No. 5). No. 5 showed a slightly higher suspension. In Nos. 6 to 12 the color and suspension gradually decreased, although the difference in suspension between the mixtures 4–0.808–50 No. 3) and 4–4–50 (No. 10) was negligible, evidently on account of the high dispersion of the lime. None of the mixtures of this series suffered decomposition within three hours.

With milk of lime the mixture 4–0.672–50 (No. 1) gave a light blue, coarse, flocculent precipitate, increasing to a full blue, gelatinous precipitate in the mixture 4–1.5–50 (No. 5), but the highest suspension was generally obtained with a 4–2–50 mixture (No. 6) or occasionally with a 4–2.25–50 mixture (No. 7). In Nos. 8 to 12 the color and suspension gradually decreased, although the difference in suspension between the mixtures 4–0.808–50 (No. 3) and 4–4–50 (No. 10) was inappreciable when the lime was properly prepared. On warm days decomposition sometimes occurred in the third hour with a 4–3–50 mixture (No. 9) and more frequently in the second or third hour with the mixtures 4–4–50 (No. 10), 4–6–50 (No. 11), and 4–8–50 (No. 12). On the average, milk of lime proved nearly as satisfactory as precipitated lime but judging from the color and suspension, was less active, far more variable, and in some instances, even erratic.

With dry, hydrated lime the full blue precipitate and the highest suspension were obtained with the mixture 4–2–50 (No. 6). The product was less efficient than milk of lime, but the mixtures 4–1–50 (No. 4) to 4–2.5–50 (No. 8) gave satisfactory results, considering the quality of the hydrate employed. Occasionally a test broke down in the second or third hour.

With all four forms of calcium hydroxide, No. 1 precipitate was of a light or greenish-blue color, and of a flocculent rather than a gelatinous character. The presence of any soluble copper was accompanied by reduced suspension. No. 2 was slightly darker in color and somewhat flocculent. Nos. 1 and 2 were rather inferior for spraying purposes. Lime in excess of the mixture 4–2–50 tends to drag down the precipitate, especially when coarse particles are present. With limewater, No. 3 was preferable, probably on account of the greater dilution.

The foregoing results conform essentially to the relative activity of the different forms of calcium hydroxide employed. Limewater is the most active, as the material is entirely soluble and partly ionized. Precipitated lime, a resultant of chemical precipitation, is finely divided and uniform and approaches limewater in activity. The activity of milk of lime varies with the quality of the quicklime and the thoroughness of slaking. The particles are naturally larger than those of precipitated lime. Commercial hydrated lime is extremely variable, both in composition and in the size and character of the particles, but it is reasonable to suppose that a high-grade product would approach freshly slaked lime in activity and assure even more uniform results in general use.

INFLUENCE OF METHOD OF MIXING

The effect of the method of mixing is important and must also be considered. There are 12 or more methods with innumerable modifications, for mixing solutions of copper sulphate and lime in suspension. At least three concentrations, i. e., concentrated, equal volume, and dilute, are feasible without subsequent dilution for each procedure, copper into lime, and lime into copper.

PROCEDURE AND RESULTS

The methods of mixing are as follows:

Method	Volume per cent	Volume c. c.
1. Concentrated copper into dilute lime	15 : 85	117 : 663
2. Equal volume of copper into lime	50 : 50	390 : 390
3. Dilute copper into concentrated lime	85 : 15	663 : 117
4. Concentrated lime into dilute copper	15 : 85	117 : 663
5. Equal volume of lime into copper	50 : 50	390 : 390
6. Dilute lime into concentrated copper	85 : 15	663 : 117
7. Concentrated copper and dilute lime poured simultaneously into a third receptacle	15 : 85	117 : 663
8. Equal volumes of copper and lime poured simultaneously into a third receptacle	50 : 50	390 : 390
9. Dilute copper and concentrated lime poured simultaneously into a third receptacle	85 : 15	663 : 117
10. Concentrated copper into concentrated lime and diluted	30 : 30 : 40	234 : 234 : 312
11. Concentrated lime into concentrated copper and diluted	30 : 30 : 40	234 : 234 : 312
12. Concentrated copper and concentrated lime poured simultaneously into a third receptacle and diluted	30 : 30 : 40	234 : 234 : 312

The volume ratios, 15 : 85 and 30 : 30 : 40, etc., are purely arbitrary, but serve to illustrate the principle involved. As in the previous experiment, rubber-stoppered museum jars were used in making the tests. The Bordeaux mixture was prepared with milk of lime from carefully slaked lime and the mixture 4–4–50 was generally employed as the basis for comparison since the mixture 4–2–50 proved less discriminating. The other forms of calcium hydroxide might also have been included, but were not considered necessary.

TABLE 2.—*Ratio of volume of suspended Bordeaux to total volume of liquid, when different methods of preparation were used*

Method No.	Bordeaux mixture, 4–4–50, first series			Bordeaux mixture, 4–4–50, second series						Bordeaux mixture, 4–2–50					
	All tests			All tests			Excluding tests that decomposed within 3 hours			All tests			Excluding tests that decompose within 3 hours		
	1 hour [a]	2 hours [a]	3 hours [a]	1 hour	2 hours	3 hours	1 hour [a]	2 hours [a]	3 hours [a]	1 hour	2 hours	3 hours	1 hour [a]	2 hours [a]	3 hours [a]
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*
1	88.2	57.9	50.3	92.2	81.2	73.5	93.1	84.5	76.8	93.8	88.3	82.6			
2	96.5	85.6	78.0	96.5	93.0	89.7				97.0	94.7	92.2			
3	97.2	89.3	81.5	96.4	93.0	89.8				97.3	95.1	92.7			
4	96.6	84.5	75.1	96.3	92.4	88.9				97.5	95.2	92.9			
5	95.9	81.3	72.1	96.7	93.4	90.3				97.7	95.7	93.5			
6	74.4	50.8	44.5	85.3	59.2	49.8	(b)	(b)	(b)	93.4	86.8	80.4			
7				95.4	90.2	85.2				95.9	92.1	88.5			
8	95.8	77.9	70.5	96.8	93.5	90.2				97.2	94.8	92.5			
9				96.9	93.6	90.4				97.3	95.4	93.1			
10	95.2	85.6	76.5	96.0	92.0	82.5	96.0	92.3	87.8	95.7	90.2	84.4			
11	93.1	74.8	62.9	96.2	91.4	81.8	96.3	92.7	88.4	95.7	85.8	76.9	95.9	90.5	85.6
12				96.2	85.7	76.0	96.5	93.2	89.5	95.2	79.1	73.3	96.0	91.5	86.8

[a] Blanks in this column indicate no decomposition. Results are as in "all tests."
[b] All decomposed.

In the first series reported the milk of lime was not as carefully screened in every case nor the mixtures as thoroughly agitated as in later practice. As a result the differences between the several members of the series are increased. Apparently all tests below 60 per cent suspension must have suffered more or less decomposition, but none of them were excluded from the average in this series. Of the nine methods tested, dilute copper into concentrated lime (No. 3) gave the best suspension, and dilute lime into concentrated copper (No. 6) the poorest; an equal volume of copper into lime (method No. 2) was preferable to an equal volume of lime into copper (No. 5); and concentrated copper into concentrated lime and diluted (method No. 10) was better than concentrated lime into concentrated copper and diluted (No. 11); but concentrated copper into dilute lime (method No. 1) was much inferior to concentrated lime into dilute copper (No. 4). From the above results it follows that suspension varied inversely with the amount of lime in solution when mixed. Copper into lime (methods Nos. 1, 2, 3, and 10) proved superior to the reverse procedure (methods Nos. 4, 5, 6, and 11), except in the case of concentrated copper into dilute lime (method No. 1), previously noted. Whether a small quantity of lime in solution favors the formation of a lower basic sulphate in the primary reaction or a basic sulphate of

a higher hydration, or whether the degree of fineness of the lime particles is the deciding factor by controlling the speed of reaction, or by reducing the drag on the precipitate when added to the copper solution, is uncertain; but the resulting gelatinous precipitate was more bulky and settled more slowly. Considering the data as a whole, method No. 3 gave the best suspension, with little choice between methods Nos. 2, 10, and 4 or between Nos. 5 and 8, while methods Nos. 11, 1, and 6 were the poorest. Method No. 3, requiring the addition of cold, sufficiently dilute copper sulphate to cold, concentrated milk of lime to make the required volume, and thorough agitation, was the most dependable, and as simple and practical a procedure as any.

With the elimination of coarse particles from the milk of lime (see p. 678), immediate and thorough agitation after mixing, and a tolerance of 1 per cent in rating, substantially uniform, average suspensions were obtained in the second 4–4–50 mixture series from dilute copper into concentrated lime (method No. 3), dilute copper and concentrated lime into a third receptacle (method No. 9), concentrated lime into dilute copper (method No. 4), equal volume of copper into lime (method No. 2), equal volume of copper and lime into a third receptacle (method No. 8), and equal volume of lime into copper (method No. 5); but inferior and more or less unstable mixtures were secured from concentrated copper and dilute lime into a third receptacle (method No. 7), concentrated copper into dilute lime (method No. 1), and dilute lime into concentrated copper (method No. 6), increasing in the order named; also, concentrated copper into concentrated lime and diluted (method No. 10), concentrated lime into concentrated copper and diluted (method No. 11), and concentrated copper and lime into a third receptacle and diluted (method No. 12), increasing in the order named. Dilute copper (methods Nos. 3 and 9) proved slightly superior to an equal volume (methods Nos. 2 and 8) and decidedly more efficient than concentrated copper (methods Nos. 1 and 7) whether poured directly into the lime or simultaneously into a third receptacle. Copper and lime poured simultaneously into a third receptacle (methods Nos. 7, 8, 9, and 12) was slightly more efficient than copper into lime (methods Nos. 1, 2, 3, and 10) except in the case of concentrated copper into concentrated lime and diluted, and concentrated copper and concentrated lime poured into a third receptacle and diluted (methods Nos. 10 and 12).

From the above it will be seen that the line of demarcation between dilute copper and an equal volume of copper and between concentrated lime and an equal volume of lime, from copper into lime or lime into copper, or both into a third receptacle, is not significant; but that the demarcation is apparent between an equal volume of copper and concentrated copper, and between an equal volume of lime and dilute lime by all three procedures, and also between copper into lime and lime into copper. The limiting factors noted in the first series of Bordeaux mixture, 4–4–50, probably prevail in the second, but with a more uniform and highly dispersed milk of lime their influence is more difficult to perceive. Possibly with a more highly dispersed milk of lime, if such could be prepared, these differences might in turn become even less perceptible. The rapidity with which the several mixtures decomposed is an additional characteristic to be

noted. A part of the tests by methods Nos. 10 and 11 broke down in the third hour, by No. 12 in the second or third hour, and by No. 1 in the second hour; and all the tests by method No. 6 in the first or second hour.

The results with 4–2–50 Bordeaux are higher and less discriminating than with 4–4–50, second series, except in methods Nos. 11 and 12, but otherwise serve to substantiate what has been said. A part of the tests by methods Nos. 11 and 12 decomposed in the second hour. The decomposition of Bordeaux mixture is manifested by a gradual loss of gelatinous character, more rapid settling, spurting of the mixture and the slow acquisition of a purplish color. The final dense aggregates are said to be sphaerocrystals. Decomposition increases with copper content, ratio of lime to copper sulphate, mixing of concentrated copper and dilute lime or of concentrated copper and concentrated lime and diluting, and with temperature, as previously shown.

RESULTS OBTAINED BY OTHER INVESTIGATORS WITH DIFFERENT METHODS OF MIXING

Other investigators (Table 3) have noted the effect of different methods of preparation on the suspension of Bordeaux mixture, and some of their results are cited although not strictly comparable. Butler (*2, p. 161*) found that a concentrated milk of lime (low amount of lime in solution) favored suspension but was not in agreement as to the relative efficiency of the several methods. Pickering (*1, p. 53*) showed that copper into lime was preferable to lime into copper. For some reason not apparent the writers obtained far better results on mixing concentrated solutions and diluting than Butler, Jones (*6, p. 91*), Pickering, and Warren and Voorhees (*20, p. 233*).

TABLE 3.—*Ratio of volume of suspended Bordeaux to total volume of liquid by other investigators* [a] *when different methods of preparation were used*

Method No.	Butler, 4-4-50		Hawkins, 4-3-50		Jones, 7.5-5-50		Pickering, 3.2-3.2-50		Warren and Voorhees, 4-4-50	
	1 hour	2 hours	1 hour	2 hours	1 hour	2 hours	1 hour	2 hours	1 hour	2 hours
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	88.5	70.9	70.3	.....	87.0	.....	98.0	90.0	68.2	.....
2	92.7	86.3	.....	.....	87.0	.....	93.0	64.0	.....	.....
3	94.8	89.5	.....	.....	.....	.....	.....	.....	.....	.....
4	92.6	85.3	80.5	.....	85.0	.....	54.0	43.0	79.5	.....
5	93.9	88.7	.....	.....	96.0	.....	69.0	54.0	.....	.....
6	91.2	82.9	.....	.....	.....	.....	.....	.....	.....	.....
7	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
8	92.0	84.6	97.1	.....	87.0	.....	.....	.....	68.2	.....
9	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
10	72.7	47.2	.....	.....	.....	.....	30.0	25.0	.....	.....
11	76.6	54.9	.....	.....	48.0	.....	27.0	22.0	22.7	.....
12	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

[a] For specific reference to the work of these investigators see "Literature cited," p. 685.

While the fungicidal activity of Bordeaux mixture is said to be inversely proportional to the amount of lime in excess of that required for complete precipitation of the copper, its efficiency as a whole is largely dependent on the degree of dispersion and other physical characteristics as determined by suspension, which is undoubtedly the simplest and most practical method of evaluation.

SUMMARY

Four forms of lime (limewater, chemically precipitated lime, milk of lime, and commercial hydrated lime) were employed in preparing Bordeaux and allied mixtures. The activity varies directly as the degree of dispersion and decreases in the order named.

The best ratios of active lime in the several forms to copper sulphate, as judged by suspension, are as follows:

Limewater	0.538 gm.–0.109 gm.–100 c. c.
Precipated lime	4 pounds–1.50 pounds–50 gallons.
Milk of lime	4 pounds–2.00 or 2.25 pounds–50 gallons.
Hydrated lime	4 pounds–2.00 pounds–50 gallons.

The best method of mixing, similarly judged, is by pouring dilute copper into concentrated lime or the two simultaneously into a third receptacle.

Some of the better grades of hydrated lime are promising substitutes for milk of lime but require soaking before being used.

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THE MORPHOLOGICAL DIFFERENTIATION OF THE PISTILLATE FLOWERS OF THE PECAN[1]

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INTRODUCTION

Most fruit trees bear blossom buds that are easily recognized during the dormant season. Such, however, is not the case with the pecan, whose fruiting habit has been little understood because of the obscurity of the pistillate buds during the dormant season. Although pruning is a major subject in the science of horticulture, no adequate system of pruning has yet been developed for the bearing pecan tree. The reason for this lies in the general lack of knowledge concerning its fruiting habit.

Woodroof (*7, p. 139*)[3] divided the buds of the pecan into two classes—catkin buds and vegetative buds. He did not, however, make clear the identification of the fruiting buds. The purpose of the present paper is (1) to show that there are four distinctly different types of terminal buds in the pecan, (2) to point out which of these are the fruiting buds, (3) to indicate the time at which the pistillate flowers begin to differentiate, and (4) to explain the process of differentiation.

The time of differentiation of the blossom buds of all cultivated fruit trees so far studied is in the growing season previous to the time of their unfolding. This fact led Goff (*3, p. 316*) to conclude that flowers were formed in the season preceding their unfolding even though their floral parts can not be distinguished in autumn. The findings of Albert (*1, p. 417*) in the case of *Robinia pseudacacia*, however, indicate that the differentiation of flower buds in early spring is entirely possible, so far as the histological characteristics which are observable under the microscope are concerned.

METHOD OF PROCEDURE

The winter buds of the pecan are protected by a dense mass of hairs which are compressed in such a manner that they become a great obstacle to the technician.

The selection of a method of histological technic which had been successfully used by previous investigators of pecan buds was limited to that employed by Woodroof, which consisted of the free-hand sectioning of green buds (*7, p. 139*). This method was tested thoroughly. It was found to be crude, but by its use it was possible to make fair sections of 40 microns in thickness. The great quantity of hairs in the winter buds caused the sections to be irregular, and the successive arranging of sections was a slow, tedious procedure.

[1] Received for publication Sept. 6, 1926; issued May, 1927.
[2] Acknowledgments are due J. N. Martin and H. W. Richey, of Iowa State College, for helpful suggestions at the beginning of the investigation.
[3] Reference is made by number (italic) to "Literature cited," p. 696.

The method was improved for rough examinations by embedding the green buds in paraffin.

A systematic effort to use the paraffin method (*2, p. 107*) resulted in the development of the acetic-anhydride method, which was successfully employed in the sectioning of the woolly winter buds. With this method there was a modification, partial removal, and unfolding of the bud hairs, thus permitting the complete infiltration of the paraffin. Moreover, the bud tissues were not so hard after dehydration as is usually the case.

Buds were collected at intervals of one week, beginning on the first Monday in September, 1925. Summer buds had been examined previous to this time to make sure that differentiation had not taken place. Ten buds were selected from different parts of the key tree, which was of the Moneymaker variety. The age of this tree was 11 years, its diameter at the base was 7 inches, its spread was 20 feet, and its height was 22 feet. As soon as differentiation began to take place in the key tree, the collections were made at three-day intervals and the studies were enlarged to include the Burkett, the Indiana, and the Halbert varieties.

After the development of the acetic-anhydride method the laboratory work resolved itself into the routine of carrying up the buds, sectioning, staining, mounting, and the examination of the sections.

A marked growth in the buds began on March 20, 1926. The embryonic shoot advanced beyond the bud hairs. By careful dissection under a binocular, it was found possible to remove all of the hairs. The dissected buds were then killed in Bouin's fluid (*2, p. 29*) and fixed in alcohol during the washing and dehydrating process.

A rotary microtome was used for sectioning. The sections were cut from 12 to 50 microns in thickness. The best results were obtained from 25-micron sections. Mayer's albumin fixative proved to be useless for these sections. Land's gum-arabic fixative (*2, p. 119*) was resorted to with success.

The technic used in fixing and floating out the ribbons consisted of smearing three drops of a 1 per cent solution of gum arabic on the slide, placing the ribbons, adding several drops of a fresh, weak solution of potassium bichromate, warming the slide high above the flame of a Bunsen burner until the ribbons floated out smoothly, then draining off the excess solutions and drying the slide in sunlight. The paraffin was removed from the slide and sections by placing them for 10 minutes in a Coplin jar of 100 per cent xylene. The slides were then passed through a 50 per cent solution of xylene in absolute alcohol to 100 per cent alcohol.

Saffranine and Delafield's haematoxylin were used both singly and as a double stain. For the alcoholic saffranine stain the slides were passed from 100 per cent alcohol through 95 per cent and 85 per cent alcohol for three minutes each before they were transferred to the stain. When the double stain was used the slides were stained in saffranine for 12 hours, passed into 50 per cent alcohol until the desired color was obtained, and then rinsed in water for 5 minutes. From water they were placed in Delafield's haematoxylin for 20 minutes, rinsed in water for 5 minutes, passed through water slightly acidulated with hydrochloric acid for 2 seconds, and rinsed again in water for 20 minutes. The slides were then passed through 50 per cent and 85

per cent alcohol for 1 minute each, through 100 per cent alcohol for 5 minutes, through 100 per cent xylene for 3 minutes, and then to balsam, after which cover glasses were applied.

As soon as the slides were dried sufficiently to prevent the slipping of the cover glasses, microscopic examinations were made, using the 8-mm. objective for preliminary studies and the 16-mm. and oil-emersion objectives for more detailed studies. As each slide was examined those sections which contained details of interest in the study were marked by means of a circle made on the cover glass with a wax pencil. This marking saved time in the final examinations and in the photographic work.

Representative slides were selected to show chronological stages in the morphological differentiation of the pistillate flowers.

TYPES OF TERMINAL BUDS

The first requirement for intelligent procedure in the study of pistillate flower buds is to ascertain exactly where these flowers occur. Woodroof (*7, p. 139*), after placing all pecan buds in two classes—catkin (reproductive) buds and vegetative buds—then pointed out that the upper serial buds are the catkin buds. It is quite true that all upper serial buds produce catkins, but all upper serial buds do not normally bear pistillate flowers. Abnormal conditions, such as the removal of certain terminal buds or the killing of terminal buds by cold, may cause lateral buds, which normally would produce catkins only, to develop pistillate flowers. Whether a pistillate flower will develop from a lateral bud under these circumstances seems to depend on some internal nutritive combination. This may be the carbohydrate-nitrogen relation of Kraus and Kraybill (*5, p. 85*). Lateral buds, which by microscopic examination are known to contain catkins, when budded into a young stock or a vigorous sprout normally produce a vegetative shoot terminated by a strictly vegetative terminal (rarely catkins develop along with the vegetative shoot). This fact has been observed even in the case of current-season buds placed in August and forced out immediately. A pistillate flower, however, is seldom if ever formed from such buds.

Pistillate flowers are usually said to originate from terminal buds. Bearing pecan trees, however, were found to have four distinctly different types of terminal buds. The first type is strictly vegetative and is the only type to be found in a young unbearing tree. It is a true terminal bud, and is distinctly different in size, shape, and structure from all other pecan buds. The second type of terminal bud is very similar in size, shape, and structure to the lateral buds. It was subtended in the growing season by a full-sized leaf. In early spring it was accompanied by a cluster of pistillate flowers which failed to develop, thus permitting the bud to occupy a strictly terminal position. Woodroof (*8, p. 678*) considers this the apical, dormant, lateral bud. From a strictly botanical standpoint this consideration is correct, but the position of the bud gives it dominance over other lateral buds and justifies its being termed a false terminal bud. The third type is a terminal bud located at the base of the peduncle of the nut cluster. During its development this bud was subtended by a full-sized leaf. It differs from the second type in

that the flower cluster set and matured a cluster of nuts. It, too, may be called a false terminal bud. The fourth type is a false terminal bud which seems to be borne on the peduncle of the nut cluster, but it was not subtended during the growing season by a fully developed

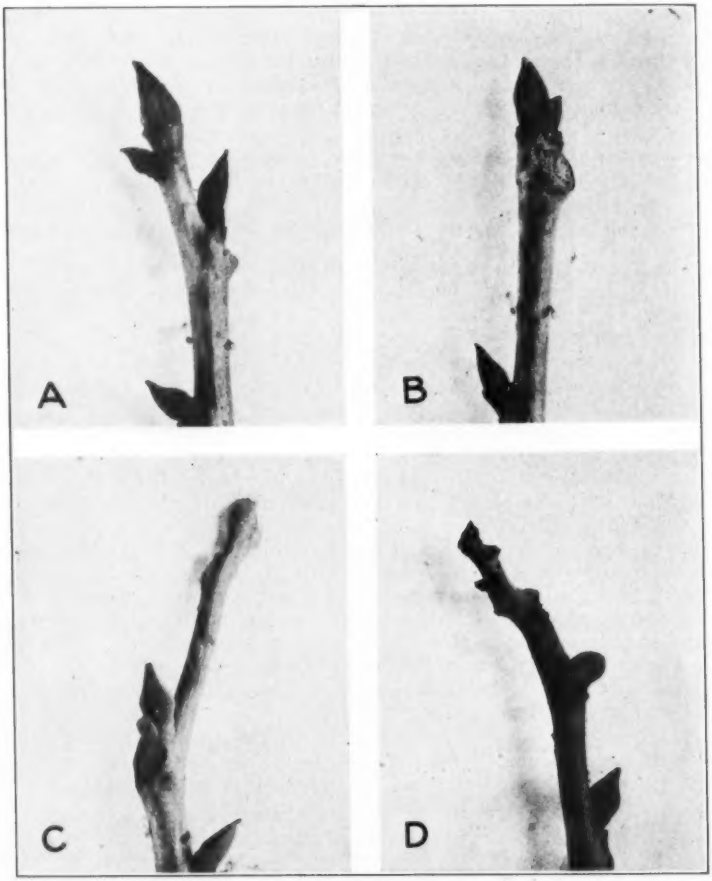


FIG. 1.—Types of terminal buds in the pecan: A, type 1, vegetative (true terminal); B, type 2, non-fruiting branch terminal (false terminal); C, type 3, fruiting-branch terminal with leaf scar (false terminal); D, type 4, fruiting-branch terminal without leaf scar (false terminal)

leaf. As many as five such buds, fully developed but without subtending leaves, have been found on a nut-cluster peduncle of the Schley. These four types of terminal buds are illustrated in Figure 1.

The relative frequency of the four types of terminal buds of the pecan tree used in this investigation is shown in Table 1.

TABLE 1.—*Frequency distribution of the four types of terminal buds on an 11-year-old pecan tree of the Moneymaker variety*

Type	Number of buds	Percentage
1, vegetable terminal	184	46
2, nonfruiting-branch terminal	76	19
3, fruiting-branch terminal with leaf	58	14.5
4, fruiting-branch terminal without leaf	82	20.5

The source of each branch which bore these terminals is shown in Table 2.

TABLE 2.—*Source of branch on which each type of terminal bud was borne*

Type of terminals on past-season branches	Kind of buds, developing branches	Number of branches developed	Number of current-season branches containing terminals of—			
			Type 1	Type 2	Type 3	Type 4
Type 1	Terminal	46	46	None.	None.	None.
	Lateral	206	116	46	18	26
Type 2	Terminal	50	None.	12	14	24
	Lateral	34	12	4	12	6
Type 3	Terminal	12	None.	2	6	4
	Lateral	30	6	8	6	10
Type 4	Terminal	None.	None.	None.	None.	None.
	Lateral	22	4	4	2	12

SOURCE OF PISTILLATE FLOWERS

That the chief source of shoots bearing type 1, vegetative terminals, is from the terminals and the laterals on a vegetative shoot is clearly shown by Table 2. It is also clearly shown that type 2 terminal buds and lateral buds near the tip of the same shoot are prolific sources of pistillate flowers. Another prolific source is the lateral buds near the tip of type 1 terminals. As a rule, fruiting branches do not originate from any lateral buds which are more than 2 inches from the terminal, regardless of its type. Not more than three pistillate buds normally develop on any one shoot. In old trees it was observed that pistillate flowers more often originate from shoots bearing type 3 and type 4 terminals, but the type 2 terminal is an important source in these trees as well.

When a pecan tree becomes 35 or 40 years of age it does not normally bear many, if any, type 1 terminals. In these trees the types 2, 3, and 4 terminals are abundant. Type 1 terminal, being the only one present in young unbearing trees, is gradually eliminated until all of the terminal buds of the tree are of the fruiting type.

This relationship of age to the source of pistillate flowers is shown in Figure 2. Since it is seldom that a branch bearing type 2, 3, and 4 terminals will produce a strictly vegetative shoot, these three types are plotted as sources of pistillate flowers; that is, fruiting buds as compared with the strictly vegetative type 1 terminal.

The identification of the pistillate flowers during the dormant season is indefinite in all features other than location. After the

buds begin to grow in spring those bearing pistillate flowers may be recognized usually by their greater length, provided they are located in the proper position. This, however, will not differentiate between the pistillate flowers originating from laterals on a vegetative shoot and vegetative shoots originating from the same source. Figure 3 illustrates the pistillate flower buds in early spring.

It has been observed that when conditions are unusually favorable to the development of pistillate flowers, even the type 1 terminals are prolific sources of them. This, however, is considered an abnormal condition.

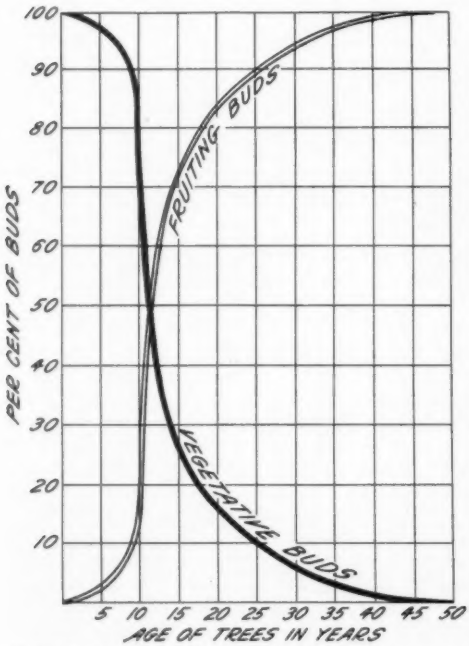


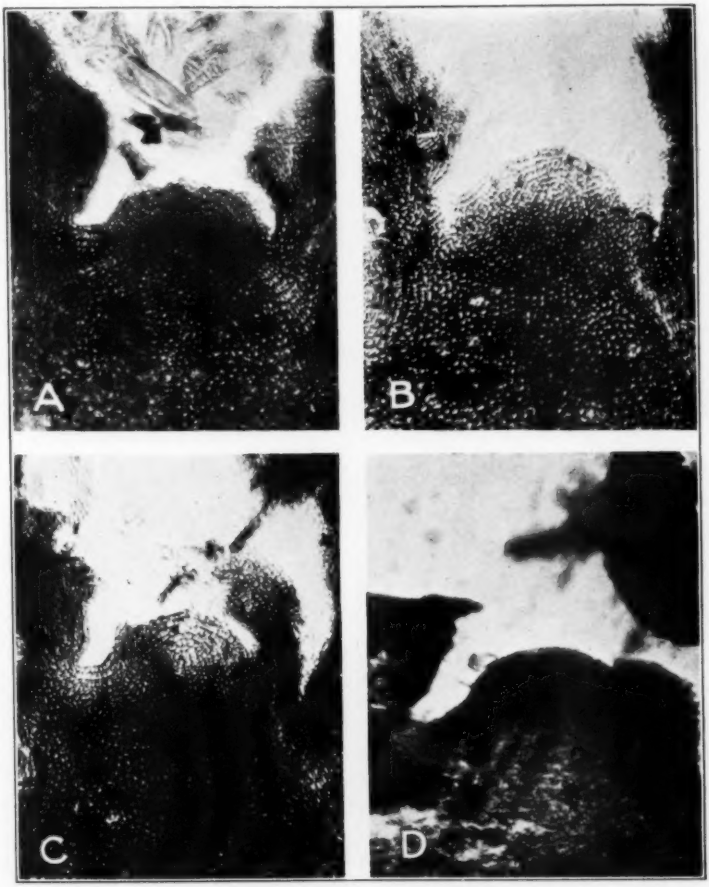
FIG. 2.—Relationship of the age of a pecan tree to the vegetative and the fruiting buds which it bears

TIME OF DIFFERENTIATION

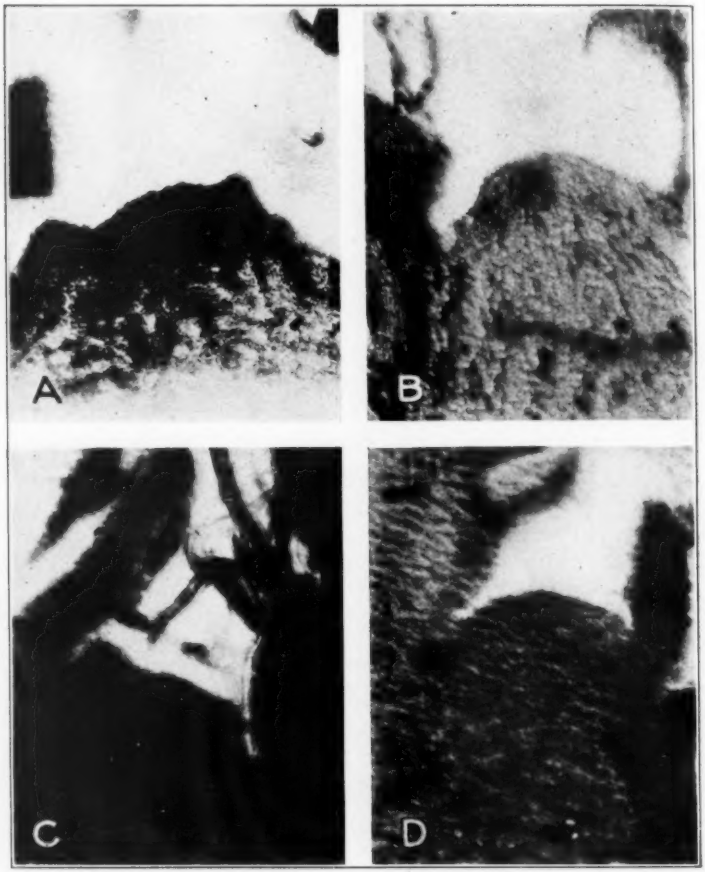
Observable differentiation in the pistillate flowers of the pecan does not take place until after growth has started in the spring. The time of differentiation will therefore vary with seasonal, climatic, and latitudinal variations.

No differentiation had begun in the Moneymaker variety at Stillwater previous to March 25, 1926 (pls. 1, 2, and 3), although on that date the buds were swelling rapidly as a result of the unusually dry and warm weather. A marked thickening of the axis, corresponding to that described by Kraus (*4, p. 5*), was noted at this time. (Pl. 3, D.) These buds, however, were all killed by a series of freezes which came afterwards. Later, basal buds which were forced out as a result of the freezing of the terminal and upper lateral buds differentiated pistillate flowers. Halbert buds procured from Ardmore, Okla., on April 5, 1926, were showing the first signs of differentiation by the production of small blisterlike protuberances on the meristem. (Pl. 4, A.)

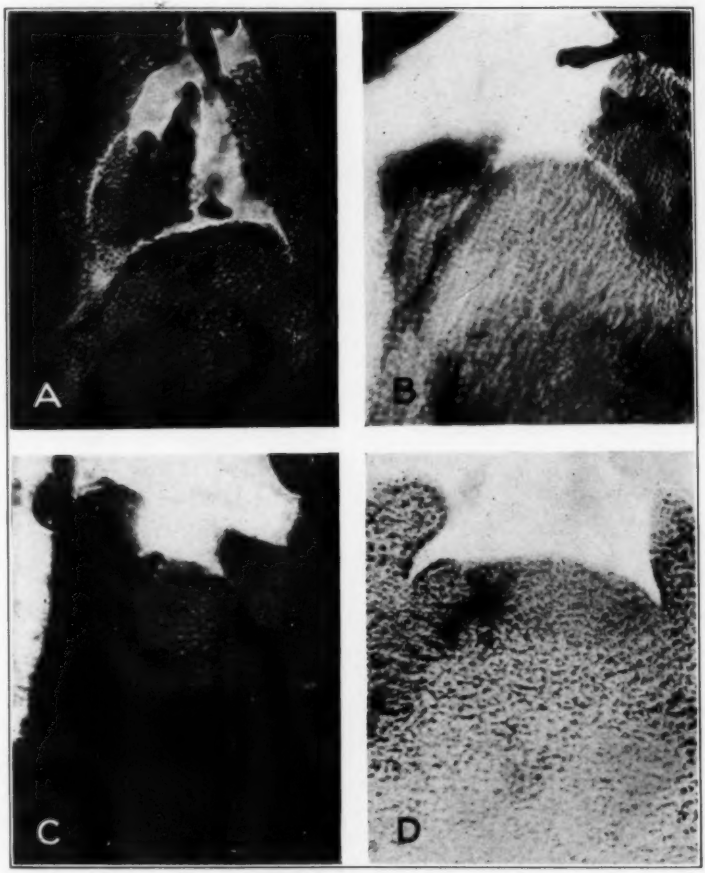
The first evidences of pistillate-flower differentiation in the Indiana and the Halbert varieties at Stillwater appeared on April 19, 1926. (Pl. 4, D.) This wide difference in the time of differentiation of the same variety in two localities of a State is in marked contrast with the close agreement in time of differentiation of pear and apricot buds in various environments (*6, p. 881*).



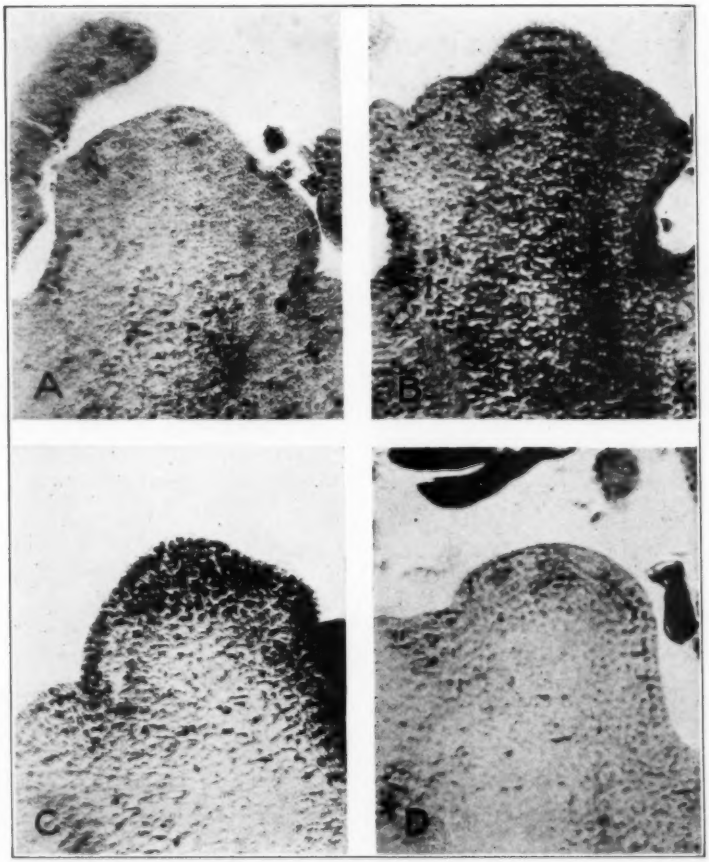
Representative section through a pistillate flower bud on different dates: A, September, 1925; B, October, 1925; C, November, 1925; D, December, 1925 (a leaf primordium is developing in this bud). All × 160



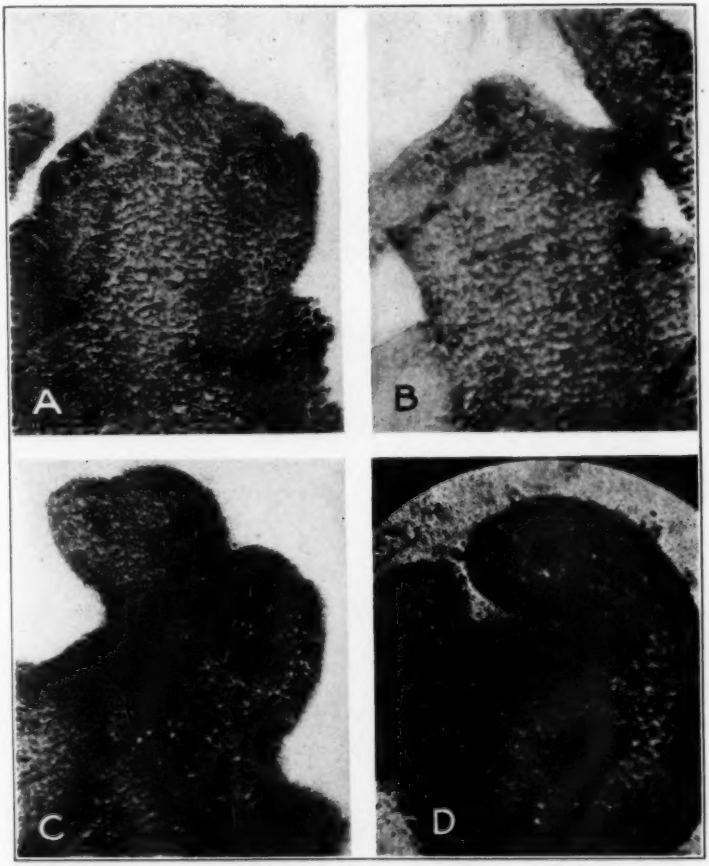
Representative section through a pistillate flower bud: A, January 1, 1926; B, January 15, 1926; C, February 1, 1926; D, February 14, 1926. All × 160



Representative section through a pistillate flower bud: A, March 2, 1926 (leaf primordium developing); B, March 7, 1926; C, March 18, 1926; D, March 25, 1926 (note the thickening of the axis; this, the first indication that differentiation is about to take place, is characteristic of the pistillate flower buds collected on this date). All × 160



Section through pistillate flower buds of four varieties of pecan: A, Halbert bud from Ardmore, Okla., showing clearly the blisterlike protuberances which are the primordia of pistillate flowers, April 5, 1926; B, a Burkett bud from Ardmore on April 9, 1926, development advanced probably five days; C, a Moneymaker bud from Ardmore, on April 9, 1926, showing the first signs of differentiation; D, an Indiana bud from Stillwater on April 19, 1926, showing the first signs of differentiation. All × 160



Section through pistillate flower buds of pecan, showing different stages of development: A, Halbert bud in first stage of development, × 160; B, Halbert bud in second stage of development, × 160; C, Halbert bud in third stage of development, × 160; D, section through a single pistillate flower in third stage of development, × 420

A Burkett bud, showing the developing flowers. Note the narrow, necklike arrangement of the fibrovascular bundles. × 216

Woodroof (*7, p. 159*) concluded, apparently without actual observation, that differentiation of pistillate flowers takes place in early spring. In his later work (*8, p. 679*) he referred to this conclusion but offered no evidence to show that the first stages of pistillate-flower differentiation had been observed.

PROCESS OF DIFFERENTIATION

Each fruit bud of the pecan bears an average of 12 leaves. During the fall and winter there may be found from 4 to 8 embryonic leaves, varying from those definitely formed to the small leaf primordia which are conical protuberances arising from the margins of the meristem.

FIG. 3.—Two pistillate flower buds developing on the stem of a branch which bore nuts the previous season. Note how much more fully developed these buds are than the other buds on the shoot

In late winter and early spring, bud growth progresses slowly. This growth, as was observed by Woodroof (*7, p. 139*), consists chiefly of the enlargement of the catkin groups. Leaf primordia continue to develop even after the casting off of the bud scales. In the spring, when growth has so far progressed that the total length of the bud is approximately 1 cm., the necessary leaves (12 as a mean) will have formed.

The leaf primordia are arranged in 3–2 cycles about the axis of the stem. The embryonic twig is therefore telescoped together in a compact manner. This arrangement is diagrammatically shown in Figure 4.

It is at this time that differentiation takes place. The pistillate flower buds indicated in Figure 3 are in the first stages of differentiation. The first observable indication of the pistillate flower is a cluster of blisterlike, blunt protuberances which form on the meristem. (Pl. 4, A.) After this initial step in differentiation, the development of the pistillate flower is greatly retarded. The leaves, however, are accelerated in their development and the internodes between them develop concurrently.

In approximately two weeks from their first appearance the blunt floral primordia become distinctly cup-shaped. The depression is formed by the rapid elevation of the rim and a corresponding latency in the development of cells near the center of the blisterlike protuberances. (Pl. 5, C and D.) As this cup continues to develop

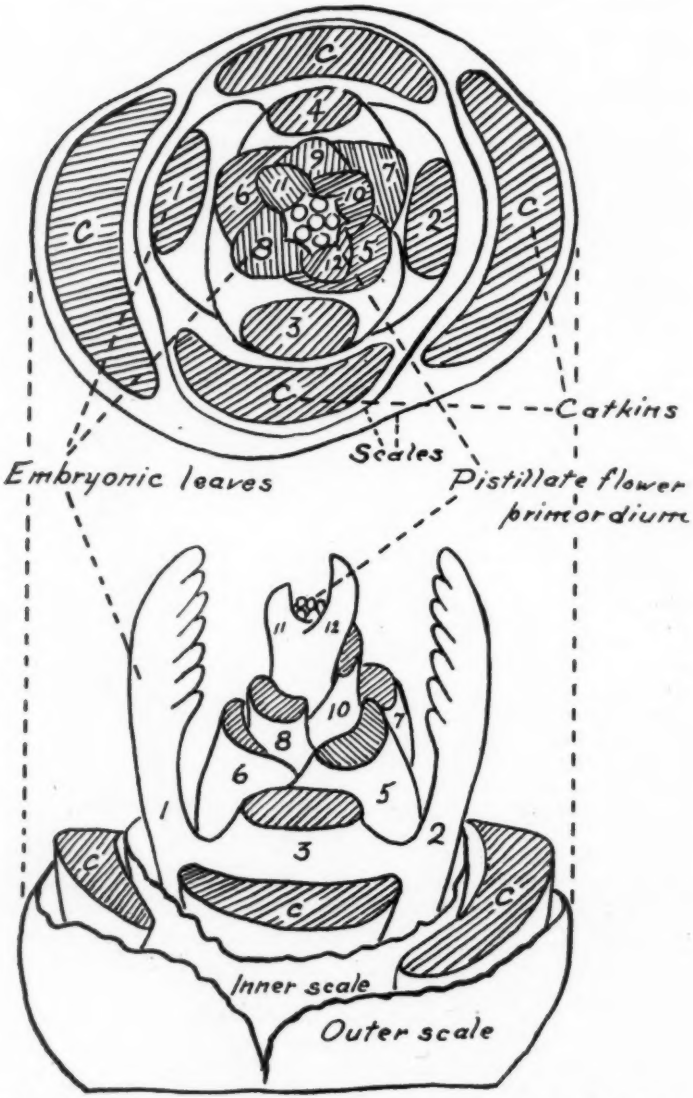


FIG. 4.—Arrangement of a pecan bud at the time that differentiation of the pistillate flowers is taking place

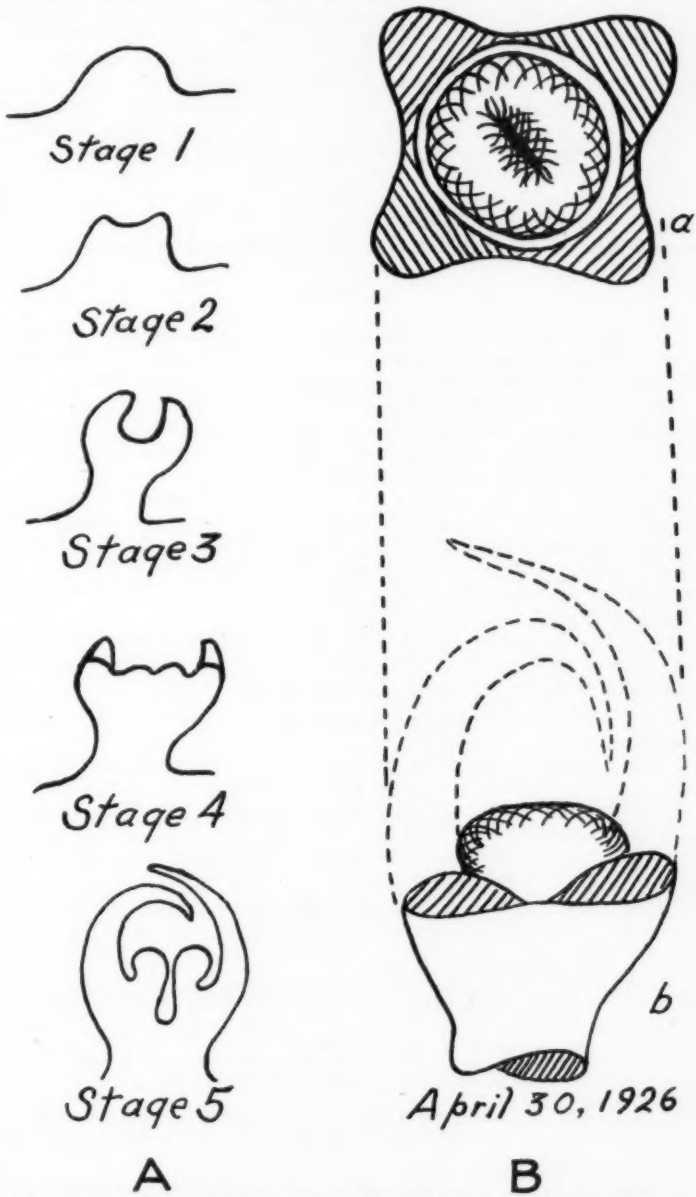


FIG. 5.—A, section through pistillate flower primordia in various stages of development; B, top view of a pistillate flower, showing the developing stigma three weeks after stage 1; *b*, side view showing the arrangement of the bracts

it becomes the hull of the embryonic nut. The cavity broadens and a second depression forms in the center, as is shown in stage 4 of Figure 5. Four points on the rim of the first cup develop more rapidly than the remaining part. These become the bracts of the calyx. As they develop they fold over and protect the stigma which forms around the second depression. This depression becomes a cavity in which the ovule develops, as is indicated in stage 5 of Figure 5.

The fibrovascular bundles of the stem continue through the developing peduncle to the pistillate flowers. A distinct necklike arrangement of these bundles is observable even in the early stages of differentiation. (Pl. 6.)

SUMMARY

There are four distinctly different types of terminal buds in the pecan. Type 1 is a true terminal; types 2, 3, and 4 are false terminals.

Normally, pistillate flowers are produced in terminal buds of types 2 and 3 and in lateral buds near the end of shoots bearing terminals types 2, 3, and 4. Lateral buds on shoots bearing type 1 terminals may produce pistillate flowers. In certain exceptional circumstances type 1 terminals may produce pistillate flowers.

Observable differentiation in the pistillate flowers of the pecan does not take place until after growth has started in the spring. The time will therefore vary with seasonal, climatic, and latitudinal conditions. Some difference has been noted between varieties, but this difference is not as great as that which results from different latitudinal locations of the same variety.

The rate of development of the pistillate flowers is much slower than that of the leaves on the same shoot. Five stages of development have been traced under the microscope.

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